

(19)



(11)

EP 2 086 322 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:
01.12.2010 Bulletin 2010/48

(51) Int Cl.:
A01N 37/22 ^(2006.01) **A01N 43/40** ^(2006.01)
A01N 61/00 ^(2006.01) **C07C 233/15** ^(2006.01)
C07C 235/74 ^(2006.01) **C07D 213/75** ^(2006.01)

(21) Application number: **07857195.7**

(86) International application number:
PCT/EP2007/060721

(22) Date of filing: **09.10.2007**

(87) International publication number:
WO 2008/049729 (02.05.2008 Gazette 2008/18)

(54) NON-STEROIDAL BRASSINOSTEROID MIMETIC

NIGHTSTEROIDES BRASSINOSTEROID-MIMETIKUM

MIMÉTIQUE DE BRASSINOSTÉROÏDE NON-STÉROÏDIEN

(84) Designated Contracting States:
**AT BE BG CH CY CZ DE DK EE ES FI FR GB GR
HU IE IS IT LI LT LU LV MC MT NL PL PT RO SE
SI SK TR**

(30) Priority: **12.10.2006 EP 06122151**

(43) Date of publication of application:
12.08.2009 Bulletin 2009/33

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Description

[0001] The present invention relates to non-steroidal mimetics of brassinosteroids. More specifically, it relates to non-steroidal monocyclic compounds, capable of rescuing the brassinosteroid receptor null mutation *bri1-116*. Preferably, said compounds are low molecular weight, monocyclic halogenated compounds.

[0002] Brassinosteroids (BRs), such as Brassinolide, 24-Epibrassinolide, 28-Homobrassinolide and Castasterone, are plant hormones involved in multiple developmental processes. Brassinosteroids are, amongst others, involved in plant growth promotion, increase in the success of fertilization, shortening the period of vegetative growth, improvement of fruit quality, increase of stress resistance and crop yield increase (Khripach *et al.*, 2000).

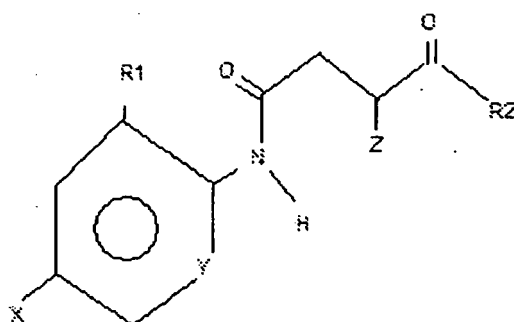
[0003] BRs are a group of naturally occurring polyhydroxy steroids. Natural BRs have essentially a common 5- α cholestan skeleton (figure 1) and their structural variations come from the kind and orientation of functionalities on the skeleton, and from variations in the B ring.

[0004] BRs exert their activity by binding to the plasma membrane receptor kinase BRI1, resulting in the activation of a signalling pathway that involves a glycogen synthase kinase-3-like kinase (BIN2) and a serine/threonine phosphatase BSU1. BIN2 negatively regulates BR signalling by phosphorylation of the transcription factors BES1 (and probably the closely related BZR1), while dephosphorylation of BES1 by BSU1 activates the transcription of BR induced genes (Clouse, 2002; Vert and Chory, 2006; Figure 2)

[0005] Due to their importance as plant growth promoting compounds, several companies developed production methods for BRs and BR analogues. Such methods have been disclosed, amongst others, in JP01075500, JP01175992 and US6667278. However, those synthetic BRs and BR analogues are generally too expensive for large scale commercial applications. Therefore, there is a clear interest in low molecular weight, non-steroidal compounds with BR activity. Non steroidal mimetics of brassinolide have been disclosed in US6667278. However, although those structures do not longer have the canonical 5- α cholestan skeleton, the molecules are rather complex and include two bicyclic subunits, each having a vicinal diol group and a polar unit. Said compounds are supposed to bind and act on the BR receptor, as is stated that the vicinal diol group and the polar group should be linked by a linking moiety such that the vicinal diol groups and polar unit are closely superimposable on corresponding functional groups in the brassinosteroid.

[0006] Using a chemical genetics approach, surprisingly we found non-steroidal, monocyclic low molecular weight compounds, having BR activity. Even more surprisingly, those compounds do not exert their activity by the BR receptor, as they can rescue the *bri1* mutation.

[0007] A first aspect of the invention is a non-steroidal, monocyclic brassinosteroid mimetic, having the formula

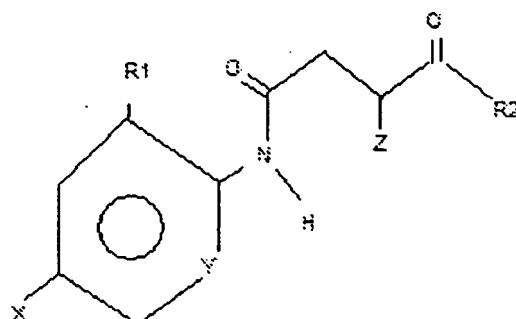


whereby (a) X represents a halogen (b) Y represent nitrogen (c) Z represents hydrogen or a positively charged nitrogen (d) R_1 represents hydrogen or a methyl group and (e) R_2 represents hydrogen, a hydroxyl, methyl or carboxy group. Preferably, said non-steroidal monocyclic brassinosteroid mimetic is 4-[(5-fluoro-2-pyridinyl)amino]-4-oxobutanoic acid. A brassinosteroid mimetic, as used here, means that the compound can be used to replace brassinosteroids such as, but not limited to brassinolide, to treat plants, in order to obtain the phenotypical effects of BR treatment. These phenotypical effects are known to the person skilled in the art, and include, but not limited to plant growth promotion, increase of yield of grain and fruit crops and induction of drought and freeze resistance.

[0008] The compound 4-[(5-bromo-2-pyridinyl) amino]-4-oxobutanoic acid - which has been disclaimed - has already been disclosed (Khimiko-Farmatsevticheskii Zhurnal, 30,11,1996 17-18 for antiviral use.

[0009] Another aspect of the invention is the use of a non-steroidal, monocyclic compound to induce brassinosteroid depending gene expression. Brassinosteroid depending gene expression as used here means both brassinosteroid depending gene induction as well as brassinosteroid depending gene repression. Brassinosteroid depending genes are under control of the BES1 and/or BZR1 transcription factors. Preferably, said genes comprise a BR response element

GGTG(T/C)G (He *et al.*, 2005; Wang *et al.*, 2006). Preferably, said non-steroidal, monocyclic compound is inducing BES1 dephosphorylation. Even more preferably, said induction of brassinosteroid depending gene expression and/or said BES1 dephosphorylation is independent from the brassinosteroid receptor BRI1. A preferred embodiment is the use of a non-steroidal, monocyclic compound according to the invention, whereby said compound has the formula



whereby (a) X represents a halogen (b) Y represent nitrogen (c) Z represents hydrogen or a positively charged nitrogen (d) R₁ represents hydrogen or a methyl group and (e) R₂ represents hydrogen, a hydroxyl, methyl or carboxy group. Preferably, said compound is selected from the group consisting of 4-[(5-fluoro-2-pyridinyl)amino]-4-oxobutanoic acid, 4-[(5-chloro-2-pyridinyl)amino]-4-oxobutanoic acid and 4-[(5-iodo-2-pyridinyl)amino]-4-oxobutanoic acid.

[0010] Still another aspect of the invention is the use of a non-steroidal, monocyclic brassinosteroid mimetic according to the invention to derive in silico compounds with a brassinosteroid mimetic activity. Indeed, by using the program ROCS (OpenEye Scientific Software, USA), as a nonlimiting example, to perform a shape-based virtual screening novel compounds that have a similar functionality can be identified. All compounds with a shape-based Tanimoto similarity higher than 0.8 can be selected. Examples of such compounds are given in the application. Another aspect of the invention is a composition for promoting plant growth, comprising a non-steroidal, monocyclic brassinosteroid mimetic according to the invention. Said composition might be an aqueous solution comprising said brassinosteroid mimetic, or a composition comprising any other suitable vector. The composition may further comprise other plant growth regulators such as auxins, cytokinins or gibberellins. Preferably said composition is comprising a non-steroidal, monocyclic compound selected from a group consisting of 4-[(5-fluoro-2-pyridinyl)amino]-4-oxobutanoic acid, 4-[(5-chloro-2-pyridinyl)amino]-4-oxobutanoic acid, 4-[(5-bromo-2-pyridinyl)amino]-4-oxobutanoic acid and 4-[(5-iodo-2-pyridinyl)amino]-4-oxobutanoic acid.

[0011] Still another aspect of the invention is a method for promoting plant growth and/or increasing crop yield by applying to the plant an effective amount of the non-steroidal, monocyclic brassinosteroid mimetic according to the invention. The promotion of the plant growth can be direct, by stimulation of the cell division, or indirect, such as by increasing abiotic stress resistance. Increase of yield can be increase of plant biomass, or increase of grain or fruit yield. Preferably, said non-steroidal, monocyclic brassinosteroid mimetic is selected from a group consisting of 4-[(5-fluoro-2-pyridinyl)amino]-4-oxobutanoic acid, 4-[(5-chloro-2-pyridinyl)amino]-4-oxobutanoic acid, 4-[(5-bromo-2-pyridinyl)amino]-4-oxobutanoic acid and 4-[(5-iodo-2-pyridinyl)amino]-4-oxobutanoic acid.

BRIEF DESCRIPTION OF THE FIGURES

[0012]

Figure 1: Chemical structure of brassinolide (BL).

Figure 2: Diagram of the brassinosteroid signal transduction pathway (according to X. Wang and J. Chory, 2006).

Figure 3: (A) Phenotypic characterization. WT plants were grown under standard conditions on agar plates. After 3 days of germination, plants were transferred to agar plates containing abscisic acid (ABA) at a concentration of 50 μ M or brassinolide (BL) at a concentration of 1 μ M. Left panel: control plant. Middle panel: ABA treated plant. Right panel: BL treated plant. **(B)** Dose response measurements of abscisic acid using lateral root formation as an indicator of brassinosteroid like activity. Error bars represent standard error of the mean.

Figure 4: Chemical structure of abscisic acid (4-[(5-bromo-2-pyridinyl)amino]-4-oxobutanoic acid).

Figure 5: Effect of brassinolide (BL) and abscisic acid (ABA) treatment on mutants. WT or mutant plants were grown under standard conditions on agar plates. After 3 days of germination,

plants were transferred to agar plates containing abrasin at a concentration of 50 μM or BL at a concentration of 1 μM .

Figure 6: Rescue of dark-grown BL mutants after treatment with 50 μM of abrasin (15).

Figure 7: Q-PCR analysis of genes involved in BR biosynthesis. WT *Arabidopsis* plants were treated with abrasin (indicated as 15) for 2 hours at a concentration of 30 μM and/or with CHX at a concentration of 30 μM and RNA was extracted from whole plants.

Figure 8: A: Western Blot analysis of the BES1 protein in 35S:BES1:GFP plants treated with BL and abrasin (indicated as 15 throughout the figure) (A); MG132 with BL or abrasin (B); a time course with abrasin (C) and a concentration gradient with abrasin (D). **B:** Abrasin and BL induce similar transcriptional regulation of the BR pathway. Transcriptional regulation (relative expression levels) of five BR-biosynthetic genes (*DWF4*, *CPD*, *ROT3*, *BR6OX1* and *BR6OX2*), five BR-regulated genes (*BRI1*, *BIN2*, *BSU1*, *BES1* and *BZR1*) and two BR-upregulated genes (*SAUR-AC1* and *BAS1*) on 3-day-old seedlings after treatment with BL (1 μM) or Abrasin (30 μM) for 6 hours compared to DMSOtreated controls (all results are means $\pm$ s.d.; individual reactions were performed in triplicate and experiments were repeated at least twice).

Figure 9: Abrasin derivatives.

Figure 10: Effect on lateral root density of abrasin (indicated as 15) derivatives. Error bars represent standard error of the mean.

Figure 11: In silico derived compounds with potential brassinosteroid mimetic activity.

Figure 12: Abrasin specifically inhibits kinase activity of BIN2 and other GSK-3-like kinases in plants. a, Autoradiography of a kinase assay with GST-BIN2 and MBP-BES1 on a concentration range of 0 to 10 μM abrasin. Coomassie staining was used as a loading control. b, Autoradiography of kinase assays with nine ASKs with MBP as a substrate in the presence or absence of 10 μM abrasin. The second member of group IV, ASK κ , had no kinase activity and was not included in the analysis. c, Autoradiography of kinase assays with AtMPK4, AtMPK6 and MBP as a substrate (left) and with AtAUR1 kinase and histone H3 as a substrate (right) in the presence or absence of 10 μM abrasin.

Figure 13: Abrasin directly interacts with BIN2. SPR sensorgrams for abrasin binding to GST-BIN2 after injection of different abrasin concentrations as indicated. The binding curves are overlaid by calculated curves resulting from the global fits of the data to a 1:1 interaction model ($\chi^2 = 0.126$). The kinetic parameters obtained for the interaction are $k_a = 407 \text{ M}^{-1}\text{s}^{-1}$, $k_d = 0.081 \text{ s}^{-1}$, $K_D = 199 \text{ }\mu\text{M}$. Reference and blank data are subtracted. The experiment was performed in triplicate.

Figure 14: Clustering of gene expression profiles by QT-clust analysis. Clusters illustrating the major patterns of the data sets. Points: BIK-30: 30 μM abrasin 30 min; BL-30: 1 μM brassinolide 30 min; DMSO-30: control DMSO 30 min; BIK-120: 30 μM abrasin 120 min; BL-120: 1 μM brassinolide 120 min; DMSO-120: control DMSO 120 min. Cluster 1: 92 genes; Cluster 2: 62 genes; Cluster 3: 28 genes; Cluster 4: 26 genes; Cluster 5: 18 genes; Cluster 6: 16 genes; Cluster 7: 9 genes; Cluster 8: 8 genes; Cluster 9: 7 genes; Cluster 10: 7 genes.

Figure 15: Phenotype rescue of *bri1-116* and *cpd* mutants. (A.) The mutant and Col-0 plants were germinated on MS+DMSO medium till day 5 and afterwards transferred on MS supplemented with DMSO, 1 μM BL or 30 μM ABRASIN until day 11. Treatment with BL rescues the *cpd* mutant phenotype but not *bri1-116* while ABRASIN rescues both mutants. (B.) 1 month-old *bri1-116* plants grown in soil were watered each day with 2 ml 300 μM ABRASIN solution which suggests ABRASIN uptake through the root. Visible elongation of the stem and petioles was observed in one week. BL: brassinolide; BIK: abrasin.

Figure 16: Rescue of *bri1-116* and *cpd* mutants grown on different concentrations of ABRASIN and BL. The plants were germinated on MS+DMSO medium till day 5 and afterwards transferred on MS supplemented with the respective concentration of ABRASIN and BL for 6 more days. Flowcytometric analysis of cotyledons and leaves was performed at the last day of treatments. (A.) Rescue of *bri1-116* mutants by ABRASIN. Increasing the ABRASIN concentration promotes cell division activation. (B.) Rescue of *cpd* mutants by ABRASIN. Increasing the ABRASIN concentration promotes cell division activation. (C.) Rescue of *cpd* mutants by BL. Both cell division and endoreduplication take place in the BL rescue strategy. BL: brassinolide; BIK: abrasin.

Figure 17: Investigation of differences in the rescue strategy of *bri1-116* and *cpd* mutants treated with ABRASIN (30 μM) or BL (1 μM). The activation of markers for auxin response (DR5) and cell division (CYCB1;1 and CDKB1;1) was followed by localization of their promoters fused to β -glucuronidase (GUS) reporter gene. The first leaf was observed for comparison between all the treatments. Samples for GUS assay were taken on day 6th (i.e. 1 day treatment), 8th (i.e. 3 days treatment), 10th (i.e. 5 days treatment). (A.) The effect of ABRASIN on the first leaf of *bri1-116* and Col-0 plants - activity localization of DR5, CYCB1;1 and CDKB1;1 promoters fused to GUS reporter gene (B.) The effect of ABRASIN on the first leaf of *cpd* and Col-0 plants - activity localization of CYCB1;1 and CDKB1;1 promoters fused to GUS reporter gene. BL: brassinolide; BIK: abrasin.

EXAMPLES

Materials and methods to the examples

Chemical genetics screening and growth conditions

[0013] A commercial 10.000 compound library (DiverSet, ChemBridge, USA) was screened for brassinosteroid related phenotypes. Three to four *Arabidopsis thaliana* (L.) Heynh. seeds were sown in 96-well filterplates (Multiscreen HTS MSBVS1210, Millipore, USA) in liquid medium derived from standard Murashige and Skoog (MS) medium in a growth chamber under continuous light (110 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ photosynthetically active radiation, supplied by cool-white fluorescent tungsten tubes; Osram) at 22°C. Three days after germination compounds were added to the 96-well plates at a final concentration of 50 μM . Plants were screened six days after germination for brassinosteroid-related phenotypes.

[0014] For further phenotypical analysis, all plants were grown on vertically oriented square plates (Greiner Labortechnik, Austria) with solid medium derived from standard Murashige and Skoog medium under the same conditions. For the hypocotyl-elongation assay, plants were grown in the dark at 22°C under the same conditions.

Abrasin, derivatives and other compounds

[0015] Abrasin and all derivative molecules were purchased from ChemBridge, USA (ChemBridge ID Abrasin: 5122035, Var2: 5122029, Var3: 5133967, Var4: 5843203, Var6: 5121777 and Var7: 5310341). Epibrassinolide (BL) and cycloheximide (CHX) were purchased from Sigma, USA. The proteasome inhibitor MG132 (Z-Leu-Leu-Leu-CHO) was purchased from BostonBiochem, USA.

Western Blotting

[0016] For protein extraction, six-day-old seedlings were grown under standard conditions as described earlier on solid medium. Plants were next soaked in liquid MS-medium supplemented with the indicated compounds (concentrations and time periods as indicated in figures). Subsequently, plants were frozen in liquid nitrogen, ground and homogenized in ice-cold homogenization buffer (25 mM Tris-HCl (pH 8), 5 mM EDTA, 1 mM β -Mercapto-ethanol, 15 mM MgCl_2 , 85 mM NaCl, 0.1 % Tween 20, 1 protease inhibitor table/150ml, Complete (Roche diagnostics, Belgium)). The homogenate was centrifuged twice (5 min, 14.000 rpm, 4°C) in an Eppendorf Centrifuge 5417. Loading buffer was added, the samples were heated for 10 min at 95°C and centrifuged. The samples were separated on a 12 % acrylamide gel or a 4-20 % gradient pre-cast gel (Bio-Rad) and blotted on nitrocellulose membranes (Hybond-C super, GE-Biosciences, UK) in 190 mM glycine and 25 mM Tris-Hcl using a mini-blotting system (Bio-Rad, USA) for 1h. Membranes were blocked overnight at 4°C in phosphate buffer with 0.1 % Tween 20 and 5 % skim milk (BD Difco, USA). For immunodetection, anti-BES1 antibodies at 1:2000 dilution and anti-GFP antibodies at 1:1000 dilution were used as primary antibody. As secondary antibody, anti-rat and anti-rabbit were used at 1:10.000 dilution. The proteins were detected by chemiluminescence (Perkin-Elmer, USA).

Real Time PCR

[0017] RNA was extracted with the RNeasy kit. Poly(dT) cDNA was prepared from 1 mg of total RNA with Superscript III reverse transcriptase (Invitrogen) and quantified on an LightCycler 480 apparatus (Roche) with the SYBR Green I Master kit (Roche) according to the manufacturer's instructions. Target quantifications were performed with specific primer pairs designed with the Beacon Designer 4.0 (Premier Biosoft International). All PCRs were performed in triplicate. Expression levels were normalized to EEF1 α and CDKA1;1 expression levels that did not show clear systematic changes in Ct value.

[0018] The primers used to quantify gene expression levels were for

BAS1: 5'-TTGGCTTCATACCGTTTGGC-3' and 5'-TTACAGCGAGTGTC AATTTGGC-3';
 BR6Ox1: 5'-TGGCCAATCTTTGGCGAA-3' and 5'-TCCCGTATCGGAGTCTTTGGT-3';
 BR6Ox2: 5'-CAATAGTCTCAATGGACGCAGAGT-3' and 5'-AACCGCAGCTATGTTGCATG-3';
 BRI1: 5'-GGTGAAACAGCACGCAAACT-3' and 5'-CACGCAACCGCAACTTTTAA-3';
 CPD: 5'-CCCAAACCACTTCAAAGATGCT-3' and 5'-GGGCCTGTGCTTACCGAGTT-3';

DWF4: 5'-GTGATCTCAGCCGTACATTTGGA-3' and 5'-CACGTGAAAACTACCACTTCCT-3'

ROT3: 5'-ATTGGCGCGTTCCTCAGAT-3' and 5'-CAAGACGCCAAAGTGAGAACAA-3';

BES1: 5'-CAACCTCGCCTACCTTCAATCTC-3' and 5'-TTGGCTGTTCTCAAACCTTAACTCG-

3';

BIN2: 5'-GTGACTTTGGCAGTGCAGAAAC-3' and 5'-CAGCATTCTCCGGGAAATAATGG-3';

BSU1: 5'-GGCGGTTTTCGTCAACAATTCC-3' and 5'-CCATCTAAACTGATCTCGGGTAAGG-3';

BZR1: 5'-CCTCTACATTCTTCCCTTTCCTCAG-3' and 5'-GCTTAGCGATAGATTCCCAGTTAGG-3';

CDKA1;1: 5'-ATTGCGTATTGCCACTCTCATAGG-3' and 5'-TCCTGACAGGGATACCGAATGC-3';

EEF1 α : 5'-CTGGAGGTTTTGAGGCTGGTAT-3' and 5'-CCAAGGGTGAAAGCAAGAAGA-3';
BK11: 5'-GCTCCGGCGTCGATGA-3' and 5'-GACGATAGTCCGGCCGTAGA-3'.

Kinase Assay

[0019] For in vitro kinase assays, MBP, MBP-BES1, and MBP-bes1 (20 ng each) were incubated with GST-BIN2 or GST-BRI1 kinase (200 ng each) in 20 μ l of kinase buffer (20 mM Tris [pH 7.5], 100 mM NaCl, and 12 mM MgCl₂) and 10 μ Ci ³²P- γ ATP. After incubation at 37°C for 40 min, the reactions were stopped by adding 20 μ l of 2 $\times$ SDS buffer and boiling at 94°C for 5 min. Proteins were resolved by a PAGE gel and phosphorylation was detected by exposing the dried gel to X-ray film. Proteins from 35S::bes1-GFP transgenic plants were used for phosphatase (CIP) treatments as described (Fankhauser et al., 1999).

Phosphatase Assay

[0020] The full-length *BSU1* and phosphatase sequences were amplified from *BSU1* cDNA with primers 5'-GTGAATTCGCTCCTGATCAATCTTATC-3' and 5'-GAGAATTCCATAAGAAGG TCATTTCGA-3' for the respective 5'-ends, and primer 5'-CGAGTCGACCCTTTATTCACTTGACTC-3' for the 3'-end. The fragments were cloned into the EcoRI/Sall sites of pMAL-C (New England Biolabs). Cultures of transformed *E. coli* BL21-CodonPlus-RIPL cells (Stratagene) were grown at 18°C in YEP medium supplemented with 0.2% glucose and 1 mM MnCl₂ until they reached an OD₆₀₀ of 0.6, induced with 40 mM IPTG, and grown for an additional 10 h at the same temperature. The fusion proteins were purified and their phosphatase activity assayed according to the manufacturer's specifications (PSP Assay System; New England Biolabs). Inhibition studies were performed using similar procedures, adding okadaic acid (Sigma) or Inhibitor-2 (New England Biolabs) to the reaction.

Shape based in silico screening

[0021] A library of compounds against which to screen was assembled from compounds of almost 40 different vendors and comprised more than 7 million original compounds.

[0022] The program ROCS (Open Eye Scientific software, USA) was used to perform shape based virtual screening. The structure of abrin was used as template against which the entire 3D-enumerated database of 11 million conformations was screened. For this purpose, the implicit Mills-Dean atom coloring scheme was used in conjunction with the standard shape-base matching of ROCS.

SPR analysis

[0023] Biacore T100 was used to analyze interaction of abrisin with BIN2. Using amine coupling, purified GST-BIN2 was immobilized in the flow cell of a Series S CM5 Sensor Chip (Research Grade, Biacore AB). HBS-EP (Biacore AB) was used as running buffer, flow rate was set at 5 μ l/min. The surface of the chip was activated by injecting a mixture of EDC (0.2 M) and NHS (0.05 M) for 10 min. Subsequently, 20 μ g/ml GST-BIN2 in 10 mM sodium acetate buffer (pH 6.0) was injected for 20 min. The immobilization level of GST-BIN2 was = 20,000 RU. The chip was then flushed with 1 M ethanolamine (pH 8.5) for 10 min to deactivate the surface. A flow cell treated with a cycle of activation and deactivation without immobilized ligand was used as a reference.

Binding experiments

[0024] Binding of abrasin to GST-BIN2 was performed in HBS-EP running buffer (Biacore AB) supplemented with 10 mM $MgCl_2$. abrasin was dissolved directly in running buffer at a concentration of 100 μ M. Different concentrations of abrasin were injected at a flow rate of 30 μ l/min over the reference and the GST-BIN2 flow cell for 90 s, followed by 180 s of buffer flow (dissociation phase). Zero concentration samples were used as blanks. The flow cell temperature was set to 25°C. Biacore T100 evaluation software (version 1.1.) was used for curve fitting, assuming a 1:1 binding model.

Microarray analysis

[0025] Col-0 seeds were germinated vertically on $\frac{1}{2}$ MS medium for 7 days under 16h light/8h dark cycles. The seedlings were overlaid with liquid $\frac{1}{2}$ MS medium containing 1 μ M brassinolide (BL, Fuji Chemical Industries, Ltd., Toyama, Japan), 30 μ M abrasin (BIK, ChemBridge Corporation) and DMSO and treated for 30 and 120 min. The shoot parts were collected for RNA isolation. All sampling points were performed in three independent experiments. RNA was extracted using RNeasy kit (Qiagen). 200 μ g total RNA per array was used to hybridise the ATH1 Affymetrix *Arabidopsis* arrays according to standard procedure. The overrepresentation analyses were performed using BiNGO software (Maere *et al.*, 2005).

Plant material and treatments

[0026] Col-0, the null BR signaling *bri1-116* (Friedrichsen *et al.*, 2000) and BR biosynthetic *cpd* (Szekeres *et al.*, 1996) mutants were subjected to phenotype rescue analysis by treatments with BL and ABRASIN. As a negative control, dimethylsulfoxide (DMSO) was used. Plants were germinated *in vitro* for 5 days on $\frac{1}{2}$ MS containing DMSO medium and from day 6th to day 11th they were transferred on medium supplemented with BL (10nM, 100nM, 1000nM), ABRASIN (5 μ M, 10 μ M, 30 μ M) or DMSO. Cotyledons and leaves were collected for flowcytometric analysis at the 11th day (i.e. 6 days of treatments). Samples for β -glucuronidase (GUS) assay were taken on day 6th (i.e. 1 day treatments), 8th (i.e. 3 days treatments), 10th (i.e. 5 days treatments). Rescue of soil-grown mutants at different growth stages was checked by watering *bri1-116* and *cpd* mutants with either BL or 2 ml 300 μ M ABRASIN per day.

Flowcytometric analysis of leaves

Samples for flow cytometric analysis were collected and analyzed as described earlier (De Veylder *et al.*, 2001)

GUS assay

[0027] GUS staining was carried out by the method described by Jefferson *et al.* (1987). Images of GUS stained plants were taken with binocular microscope (MZ16, Leica) and Nikon camera.

Example 1: identification of a monocyclic brassinosteroid mimetic

[0028] Using a chemical genetics approach, a commercial 10.000 compound library was screened for molecules, which exert a brassinosteroid-like phenotype on young *Arabidopsis thaliana* seedlings, in order to further elucidate the BR-signalling pathway. One compound (4-[(5-bromo-2-pyridinyl)amino]-4-oxobutanoic acid), designated abrasin (in the figures indicated as I5 or BIK), was identified which strongly induced elongation of leaves, petioles and the hypocotyl in a dosage-dependent manner. Furthermore, root elongation and lateral root development was inhibited with an EC_{50} -value of 20 μ M (figure 3). The chemical structure of this small molecule however showed no resemblance to the steroid-structure of known brassinosteroids (figure 4). Testing derivative molecules using SAR (Structure Activity Relationship)-data showed that altering the general structure of abrasin abolishes its activity, implying that the entire molecule

is necessary for activity. Only minor changes, like bromine to chlorine, were able to change the potency without losing activity.

Example 2: abrasin is bypassing the *bri-1* brassinosteroid receptor mutation

[0029] To determine whether the compound acts in the brassinosteroid signalling cascade, the effect on known brassinosteroid mutants was examined. Mutants in BR-biosynthesis (*cpd*, *det2-1*) and perception (*bri1-116*, *bri1-301*) are known to show a dwarfed phenotype. Addition of brassinolide (BL) rescues the *cpd* and *det2-1* mutants, but not the *bri1* receptor mutants. When grown on medium supplemented with abrasin all mutant lines, including the *bri1* receptor mutants, showed an elongated phenotype (figure 5). Furthermore, when grown in the dark, the *cpd*, *det2-1*, *bri1-116* and *bri1-301* mutants have short hypocotyls compared to wild type plants. When grown in the dark on medium supplemented with the compound, all mutant lines had hypocotyls of normal length (figure 6). These data indicate that both light and dark-grown mutant phenotypes are completely rescued to wild type by abrasin. Taken together, these data suggest that the compound interferes rather with brassinosteroid signalling downstream of BRI1 than with biosynthesis or perception.

Example 3: abrasin is inducing the brassinosteroid signalling cascade downstream the receptor

[0030] Also at the transcriptional level, a number of significant changes are invoked by abrasin treatment. Downstream of the BRI1 receptor, all genes are upregulated, suggesting an activation of the pathway. Cycloheximide (CHX) was able to induce some of these genes, but the effect of BL or abrasin treatment was not altered, which implies that the effects of abrasin are primary responses. Furthermore, all genes involved in biosynthesis and perception are regulated in the same way as a BL-treatment, indicating that this is a secondary effect caused by the activation of the signalling cascade.

[0031] Downstream of BRI1, the nuclear BES1 protein plays a central role in a phosphorylation dependent mechanism. Recent evidence showed that phosphorylation by the BIN2 kinase leads to inhibition of the DNA-binding ability on the BR-responsive target promoters as well as inhibition of transcriptional activity through impaired multimerization. Dephosphorylation of BES1 by the serine/threonine phosphatase BSU1 on the other hand, induces the BR-response. Because of this important role of the BES1 phosphorylation state, the effect of abrasin was compared to that of a BL treatment (figure 8 A). When grown on control medium, there is more phosphorylated BES1 protein present than its non-phosphorylated form. Addition of BL induces more non-phosphorylated protein. At low concentrations of abrasin of 5 to 10 μ M, a shift towards non-phosphorylated BES1 is observed. At high concentrations of 50 μ M however the total amount of BES1 protein is also reduced. Moreover, the observed shift towards the non-phosphorylated protein occurs very rapidly within 30 minutes after addition of abrasin to the medium. This correlates with the observation made before that the effect of abrasin is a primary response.

[0032] In its phosphorylated form, BES1 is thought to be degraded by the 26S proteasome. A treatment with the proteasome inhibitor MG132 however, revealed that when protein degradation is inhibited, no shift in the ratio between the phosphorylated and the dephosphorylated form is observed. However, there is an increase in the total amount of BES1. Our results support the recent view that this regulation of protein levels is not a primary response to BRs, nor a requirement for BR signalling. The reduction in protein level of BES1 is however also observed in *bin2-1* mutants compared to wild type plants after BL treatment. This indicates that abrasin, like BL, specifically inhibits BIN2 but in an even stronger manner.

[0033] To determine whether abrasin induces the BL-type growth by controlling the same subset of BR target genes, we analyzed the effect of abrasin treatment on the RNA levels of BR feedback-regulated biosynthetic genes (*DWF4* - Choe et al 1998; *CPD* - Szekere et al., 1996, *ROT3* - Tanaka et al., 2005, *BR6OX1* - Shimada et al., 2003 and *BR6OX2* - Shimada et al., 2003), genes encoding BR signaling components (*BRI1* - Clouse et al, 1996, *BIN2* - Li and Nam, 2002; Li et al., 2001, *BSU1* - Mora-Gracia et al., 2004, *BES1* - Li and Deng, 2005 and *BZR1* - Li and Deng, 2005) and BR-induced genes (*SAUR-AC1* - Vert et al., 2005 and *BAS1* - Neff et al., 1999). For all genes, the expression profiles resembled closely those of BL treatment (Figure 8 B), indicating that abrasin promotes a growth response harboring a BL signature through a common transcriptional growth-regulatory module.

Example 4: structure-function analysis of abrasin; isolation of alternative monocyclic brassinosteroid mimetics.

[0034] Several structural variants of abrasin were tested on their effect on the lateral root formation, as an indication of their brassinosteroid like activity. The compounds are listed in figure 9. All tested derivatives have completely abolished the activity, except variant 2 and variant 7 (Figure 10). Halogenation of the ring structure seems to be critical for the function; the activity is decreasing in the series F - Cl - Br - I, with the highest activity for the fluoro derivative. The nitrogen

in the aromatic ring contributes to some extent to the activity.

Example 5: in silico derivation of alternative brassinosteroid mimetics.

[0035] Based on the structure of abrasin, using a shaped based screening, several alternative compounds with potential brassinosteroid mimetic activity were derived (Figure 11)

Example 6: Abrasin inhibits BIN2 kinase activity

[0036] We next examined whether abrasin interferes with the activity of the BIN2 kinase by performing *in vitro* kinase assays. Abrasin strongly reduced BIN2 kinase activity towards its substrate BES1 in a dose-dependent manner (Fig. 12a). The inhibitory effect of abrasin was already apparent at concentrations lower than 2.5 μ M and was dramatic at 10 μ M. To demonstrate that abrasin interacts directly with BIN2, we performed surface plasmon resonance (SPR) experiments. GST-BIN2 was immobilized on a sensor chip via amine coupling and increasing concentrations of abrasin were injected over the sensor surface.

[0037] Abrasin interacted with immobilized GST-BIN2 in a dose-dependent manner (Fig. 13), with a clear response starting from a concentration of 10 μ M. In summary, these observations combined with expression and mutant analyses allow us to conclude that BIN2 represents a direct target of abrasin.

[0038] Besides BIN2, nine additional GSK-3 kinases (also designated ASKs for *Arabidopsis* SHAGGY-related kinases) divided into four subgroups (I - IV) have been identified in *Arabidopsis* (Jonak and Hirt, 2002; Yoo et al. 2006). To determine the specificity of abrasin, its effect on the kinase activity of all ASKs was analyzed with myelin basic protein (MBP) as a general substrate. Abrasin strongly inhibited the activity of the closely related groups I and II (Fig. 12b) with some residual activity (6-8 %) for group I kinases and total inhibition (1-2 % residual activity) for members of group II including BIN2. Surprisingly, one member of group III, ASK θ , was moderately inhibited (20% residual activity), whereas the activity of the other member, ASK β , was not affected by abrasin. The major difference in protein sequence between ASK θ and ASK β is localized at the N- and C-terminus (Jonak and Hirt, 2002), suggesting that N- or C-terminal residues might be crucial in determining the specificity of abrasin. Furthermore, abrasin had no effect on the activity of three unrelated *Arabidopsis* Ser/Thr kinases (AtMPK4, AtMPK6 and AtAUR1; Fig. 12c). These data indicate that the activity of abrasin is GSK-3 specific with additional specificity for certain subgroups. Currently, only members of group II GSK-3 kinases have been shown to be implicated in BR signaling (Vert and Chory, 2006, Jonak and Hirt, 2002). Interestingly, abrasin also inhibits the kinase activity of group I and ASK θ *in vitro*. However, it still remains to be demonstrated that these ASKs play a role in BR signaling besides the members of group II.

Example 7: Microarray analysis after brassinolide or abrasin treatment

[0039] We performed a microarray analysis using the *Arabidopsis* whole genome chip (Affymetix) to determine whether abrasin activated the expression of BR-inducible genes at transcriptional level. Wild-type Col-0 seedlings were exposed to 1 μ M BL, 30 μ M ABRASIN and DMSO (mock-treatment) for two time points (30 min and 120 min) and the shoot parts were collected for RNA isolation. The analysis of the variance of the normalized gene expression data took in account the variability parameters affecting the expression level: type of hormone treatment, duration of treatment and the interaction between these two. Of the nearly 23000 genes on the chip, 272 genes gave signals that were significantly above the background level in all samples at a high stringency mode (p-value of 0.05 and minimal fold change 2). Next, a subset of well-represented Gene Ontology (GO) terms (BiNGO) was used to identify functional trends in the 272 responsive genes. This analysis showed that genes encoding proteins involved in BR metabolism, BR biosynthesis, hormone mediated signaling and transcription were significantly enriched consistent with the role of BL and ABRASIN in BR signal transduction cascade.

[0040] Interestingly genes expressed in response to auxin and abiotic stimuli were also overrepresented.

[0041] Quality threshold (QC) clustering divided the significantly modulated genes into 9 clusters containing genes that shared similar expression patterns and cluster 10 containing the remaining genes (Figure 14, Table 1). The largest Cluster 1 (Table 1) contained 92 genes that were specifically and faster, within 30min down-regulated by both BL and ABRASIN but somehow strongly affected by ABRASIN. 30% of those genes were previously reported to be down-regulated by BL in the global microarray analysis performed by Nemhauser *et al.*, (2006). Consistent with the negative feedback regulation model of BR biosynthesis (Mathur *et al.*, 1998), the expression of BR biosynthesis genes (CPD, DWF4, BR6OX2) was down-regulated in Cluster 1. Both ABRASIN and BL treatment significantly down-regulated genes involved in the auxin pathway e.g. PIN7, IAA29 and IAA2 as previously reported (Mussig *et al.*, 2002; Goda *et al.*, 2002, 2004).

[0042] In Cluster 3 (Table 1), 28 genes were found to be up-regulated early by BL and later by ABRASIN. This cluster was enriched in genes previously referred to as early auxin-inducible genes from the SAUR family (SAUR-AC1, SAUR14,

SAUR10 and SAUR16). Interestingly those genes were induced by BL as fast as 30 min treatment whether ABRASIN had an effect only after 2 hours. This is consistent with previous microarray studies showing that BL induces the expression of the auxin inducible SAUR, GH3 and IAA gene families, (Goda *et al.*, 2004, Nemhauser *et al.*, 2004, 2006) in a period of 30 to 60 min.

[0043] Cluster 2 (Table 1) was enriched in genes mainly early up-regulated by the ABRASIN. However from those genes 70% were up-regulated and 30% not affected or even down-regulated later by the BL treatment. In Cluster 4 (Table 1) 26 genes were up-regulated by ABRASIN but either not changed or slightly down-regulated by BL. Based on the general expression patterns we can assume that Clusters 2 and 4 are enriched in ABRASIN up-regulated genes. ABRASIN but not BL induces the expression of 4 WRKY-family of transcription factors (WRKY15, WRKY53, WRKY33 and WRKY6), 3 DOF-type zing finger domains containing proteins (At5g60200, At2g37590, At2g28510) 2 lectin receptor-like kinases (At4g02410, At5g60270), 2 U-box domain containing proteins (At1g66160, At3g49810) and previously described HAESA (Jinn *et al.*, 2000) and HAESA-like (At5g25930) LRR-type receptor-like kinases. *Arabidopsis* WRKY proteins comprise a family of plant specific zinc-finger-type transcription factors involved in the regulation of gene expression during pathogen defense, wounding and senescence (Eulgem and Somssich, 2007). In addition to regulating the expression of defense-related genes, WRKY transcription factors have also been shown to regulate cross-talk between jasmonate- and salicylate-regulated disease response pathways (Li *et al.*, 2004). DoF proteins are members of a major family of plant transcription factors associated with plant-specific phenomena including light, phytohormone and defense responses, seed development and germination (Yanagisawa, 2002). Function of HAESA was also implicated in floral organ abscission (Jinn *et al.*, 2000). Although BRs were recently implicated in plant immunity and cell death (Kemmerling *et al.*, 2007; Chinchilla *et al.*, 2007), none of the ABRASIN specific proteins was shown to function in BR depended fashion. Cluster 5 (Table 1) was enriched in genes fast down-regulated by ABRASIN from which around 50% were later affected by BL. To some extent this cluster overlapped with Cluster 1. ABRASIN specific responses (Clusters 2, 4 and 5) were anticipated based on the observations that ABRASIN was able to inhibit the activity of not only BR specific group II GSKs in *Arabidopsis* but also group I GSKs and one member of group III.

[0044] Interestingly, Cluster 6 (Figure 14; Table 1) contained genes that were fast up-regulated by BL whether ABRASIN treatment affected them both very weakly and late or had an opposite effect. This cluster was significantly enriched in early auxin-responsive genes from the SAUR family similarly to Cluster 3 and genes involved in stomata patterning and differentiation (Nadeau and Sack, 2002; Hara *et al.*, 2007). Whereas BL initiated the expression of the auxin inducible genes within 30 min consistent with previous reports (Goda *et al.*, 2002, 2004), ABRASIN had only later and less strong effect on them. Recently a synergistic interaction between the brassinosteroid and auxin pathways was suggested based on shared target genes from their microarray data. In addition a model was proposed where both pathways converge at the level of transcriptional regulation of target genes with common regulatory elements (Nemhauser *et al.*, 2004). Our observations however suggest that the transcriptional initiation of auxin genes was not solely a result of the inhibition of BIN2 and BIN2-like GSKs. Whereas the activation of auxin responsive genes was dependent on BRI1, direct BIN2 inhibition did not result in fast auxin responses suggesting that auxin responses are result of yet unknown, BIN2 independent pathway. Interestingly, ABRASIN was able to initiate auxin responses later possibly by affecting the negative feedback loop on the BR biosynthesis. Clusters 7, 8 and 9 covered genes with more complex expression patterns.

Example 8: ABRASIN rescue experiments

[0045] *bri1-116* null mutant (Friedrichsen *et al.*, 2000) is deficient for the BR receptor, BRASSINOSTEROID INSENSITIVE 1 (BRI1) resulting in severe dwarf phenotype similar to the BR-biosynthetic null mutant, *cpd* (Szekeres *et al.*, 1996). While brassinolide (BL), the most potent BR rescued the dwarf stature of the *cpd* to a wild-type, *bri1-116* was insensitive and therefore unaffected by BL. Further we followed the effect of ABRASIN on BR mutants. *bri1-116*, *cpd* and Col-0 plants were grown *in vitro* for 5 days so the mutant phenotype was distinguishable from the wild-type (both mutants were maintained in hemizygous state). The homozygous mutants and the wild-type were then transferred to ½ MS medium containing 1 μM BL or 30 μM ABRASIN and further grown for another 6 days. Treatment with ABRASIN rescued the phenotypes of both *bri1-116* and *cpd* mutants to the wild-type (Figure 15). Phenotypic changes were observed 1 day after the treatment (day 6) and the effect increased within the next days (day 11, Figure 15A).

[0046] We next aimed to rescue soil-grown mutants, *bri1-116* and *cpd* by watering them with either BL or ABRASIN solutions at different growth stages. In trial experiments *bri1-116* and *cpd* mutants were watered with 2 ml 300 μM ABRASIN per day. This treatment was sufficient to slightly change the phenotype of 1 month-old *bri1-116* plants in a week (Figure 15B). These results showed that ABRASIN can rescue both BR perception and biosynthesis mutants.

Example 9: ABRASIN rescues the leaf growth defects in *bri1-116* and *cpd* by inducing cell proliferation.

[0047] We further investigated what are the cellular bases of the ABRASIN effect and if the mechanism by which ABRASIN and BL rescued the BR mutants was the same. We first tested different ranges of BL and ABRASIN concen-

trations in *in vitro* growth experiments on *bri1-116* and *cpd* mutants. At the 11 day, i.e. 6 days of treatments, the results were compared. It was demonstrated that 10nM BL and 10 μ M ABRASIN were sufficient to rescue the mutant phenotypes (Figure 16). In parallel cotyledons and leaves from *bri1-116* and *cpd* mutants were collected following each treatment and analyzed for DNA content by flowcytometry. The data demonstrated that ABRASIN treatment significantly increased the 2C and 4C content and completely suppressed the 16C content even at low concentrations for both *bri1-116* and *cpd* leaves. These results might suggest that ABRASIN increases the cell division activity in the treated leaves (Figure 16A and B). Interestingly ABRASIN had effect only on leaves as the values for cotyledons did not differ from the untreated controls. Although BL treatment rescued the *cpd* leaves it did not significantly change the DNA content distribution (Figure 16C).

[0048] To examine what are the molecular bases of the different ABRASIN and BL effects, cell division (*CYCB1;1*, *CDKB1;1*) and auxin response (*DR5*) markers were introduced into *bri1-116* and *cpd* mutants and compared with the wild-type. The activation of the markers was detected by localization of the activity of their promoters fused to β -glucuronidase (GUS) reporter gene in the leaf 1 and 2 (Figure 5). Samples for GUS assay were taken at day 6th (i.e. 1 day treatment), 8th (i.e. 3 days treatment), and 10th (i.e. 5 days treatment). The cell division marker, *CYCB1;1* was highly expressed in young leaves reflecting active cell proliferation (Figure 17A and B). In all cases ABRASIN treatment increased the *CYCB1;1* expression. *CDKB1;1* was used as a dual marker as its expression marked not only dividing cells but also differentiated guard cells and stomatal precursor cells (Boudolf *et al.*, 2004). Treatment with ABRASIN activated *CDKB1;1*-GUS predominately in the leaf vasculature whereas treatment with BL induced only stomata specific expression (see days 8 and 10, Figure 17A and B).

[0049] These results support the observation that ABRASIN induced strong cell division activity while the effect of BL was more related to cell differentiation.

[0050] Auxin response genes were induced by both ABRASIN and BL treatments and auxin has been implicated in leaf development (Mattsson *et al.*, 2003; Scarpella *et al.*, 2006). We next examined the auxin distribution in early leaf primordial *bri1-116* and *cpd* mutants and the ability of the synthetic auxin inducible promoter *DR5* to respond to ABRASIN and BL treatment. In *bri1-116* leaves the auxin levels seemed to be lower which was previously shown for weaker *bri* mutants (Bao *et al.*, 2004). 1 day of ABRASIN treatment did not significantly increase the *DR5* activity in the leaves but the auxin levels in both *bri1-116* and wild type leaves were increased after longer treatment (Figure 17A). This preliminary observation suggested that cell division activity caused by the ABRASIN treatment is not a solely result of an enhanced auxin response.

Table 1. Fold-change of genes following exposure to BL and ABRASIN (BIK) treatment.

Cluster	Affymetrix no.	Accession no.	Annotation	30 min		120 min	
				BIK	BL	BIK	BL
1	260655_at	AT1G19320	pathogenesis-related thaumatin family protein	0,52	0,81	0,50	0,38
1	258100_at	AT3G23550	MATE efflux family protein	0,67	0,84	0,38	0,31
1	250752_at	AT5G05690	cytochrome P450 90A1 (CYP90A1) (CYP90) (CPD)	0,63	0,93	0,29	0,41
1	247478_at	AT5G62360	invertase/pectin methylesterase inhibitor family protein	0,52	0,92	0,25	0,45
1	255942_at	AT1G22360	UDP-glucuronosyl/UDP- glucosyl transferase family protein	0,82	0,94	0,32	0,29
1	259373_at	AT1G69160	expressed protein	0,67	1,06	0,33	0,59
1	246580_at	AT1G31770	ABC transporter family protein	0,91	0,99	0,50	0,64
1	246735_at	AT5G27670	histone H2A	0,79	0,86	0,41	0,50
1	253812_at	AT4G28240	wound-responsive protein-related	0,49	0,77	0,33	0,41
1	262830_at	AT1G14700	purple acid phosphatase	0,53	0,73	0,47	0,59
1	259683_at	AT1G63050	membrane bound O-acyl transferase (MBOAT) family protein	0,75	0,98	0,27	0,40

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(continued)

	Cluster	Affymetrix no.	Accession no.	Annotation	30 min		120 min	
					BIK	BL	BIK	BL
5	1	264900_at	AT1G23080	auxin efflux carrier protein (PIN7)	0,81	0,89	0,33	0,48
	1	251321_at	AT3G61460	zinc finger (C3HC4-type RING finger) family protein (BRH1)	0,43	0,78	0,32	0,43
10	1	252178_at	AT3G50750	brassinosteroid signalling positive regulator-related (BEH1)	0,63	0,99	0,31	0,35
	1	250327_at	AT5G12050	expressed protein	0,52	0,73	0,27	0,34
15	1	261400_at	AT1G79630	protein phosphatase 2C family protein	0,62	0,78	0,35	0,37
	1	261292_at	AT1G36940	expressed protein	0,45	0,89	0,32	0,38
	1	254810_at	AT4G12390	invertase/pectin methylesterase inhibitor family protein	0,75	0,96	0,44	0,66
20	1	247191_at	AT5G65310	homeobox-leucine zipper protein 5 (HB-5) / HD-ZIP transcription factor 5	0,90	0,91	0,20	0,27
	1	262500_at	AT1G21760	F-box family protein	0,76	1,02	0,46	0,60
25	1	245987_at	AT5G13180	no apical meristem (NAM) family protein	0,77	1,07	0,39	0,50
	1	250569_at	AT5G08130	basic helix-loop-helix (bHLH) family protein (BIM2)	0,76	0,79	0,37	0,42
30	1	250248_at	AT5G13740	sugar transporter family protein	0,81	0,77	0,23	0,36
	1	262951_at	AT1G75500	nodulin MtN21 family protein	0,67	0,91	0,37	0,56
	1	245761_at	AT1G66890	expressed protein	0,65	0,72	0,14	0,34
35	1	245362_at	AT4G17460	homeobox-leucine zipper protein 1 (HAT1) / HD-ZIP protein 1	0,29	0,54	0,11	0,19
	1	247077_at	AT5G66420	expressed protein	0,92	1,04	0,42	0,43
40	1	259596_at	AT1G28130	encodes an IAA-amido synthase	0,64	0,85	0,40	0,28
	1	266363_at	AT2G41250	haloacid dehalogenase-like hydrolase family protein	0,82	0,91	0,33	0,44
45	1	253662_at	AT4G30080	transcriptional factor B3 family protein	0,89	0,94	0,41	0,49
	1	253062_at	AT4G37590	phototropic-responsive NPH3 family protein	1,04	0,90	0,43	0,53
	1	253351_at	AT4G33700	CBS domain-containing protein	0,73	0,81	0,38	0,51
50	1	259848_at	AT1G72180	leucine-rich repeat transmembrane protein kinase	0,54	0,87	0,11	0,22
	1	265511_at	AT2G05540	glycine-rich protein	0,71	0,92	0,54	0,46
55	1	253423_at	AT4G32280	auxin-responsive AUX/IAA family protein (IAA29)	0,32	0,66	0,13	0,22

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(continued)

	Cluster	Affymetrix no.	Accession no.	Annotation	30 min		120 min	
					BIK	BL	BIK	BL
5	1	256598_at	AT3G30180	cytochrome p450 enzyme	0,09	0,31	0,06	0,07
	1	267628_at	AT2G42280	basic helix-loop-helix (bHLH) family protein	0,67	0,98	0,28	0,41
10	1	265245_at	AT2G43060	expressed protein, similar to cDNA bHLH transcription factor	0,31	0,25	0,25	0,36
	1	245319_at	AT4G16146	expressed protein	0,91	0,82	0,49	0,55
	1	245784_at	AT1G32190	expressed protein	0,73	1,00	0,07	0,11
	1	253751_at	AT4G29070	expressed protein	0,78	0,90	0,50	0,74
15	1	262635_at	AT1G06570	4-hydroxyphenylpyruvate dioxygenase (HPD)	0,91	0,87	0,29	0,27
	1	252890_at	AT4G39400	brassinosteroid insensitive 1 (BRI1)	0,71	0,96	0,46	0,57
20	1	247880_at	AT5G57780	expressed protein	0,35	0,41	0,13	0,12
	1	246284_at	AT4G36780	brassinosteroid signalling positive regulator-related	0,49	0,71	0,03	0,09
25	1	266591_at	AT2G46225	Encodes a subunit of the WAVE complex	1,15	0,97	0,58	0,47
	1	257374_at	AT2G43280	far-red impaired responsive family protein	0,78	0,84	0,48	0,51
30	1	257766_at	AT3G23030	auxin-responsive protein / indoleacetic acid-induced protein 2 (IAA2)	0,55	0,66	0,25	0,39
	1	261456_at	AT1G21050	expressed protein	0,32	0,72	0,14	0,22
	1	257855_at	AT3G13040	myb family transcription factor	0,93	0,84	0,48	0,54
35	1	256960_at	AT3G13510	expressed protein	0,85	1,06	0,42	0,83
	1	253247_at	AT4G34610	homeodomain-containing protein	1,02	0,86	0,41	0,37
	1	267305_at	AT2G30070	potassium transporter (KUP1)	0,61	0,81	0,22	0,38
40	1	245075_at	AT2G23180	cytochrome P450, putative	0,67	1,10	0,36	0,42
	1	257858_at	AT3G12920	expressed protein	0,45	0,81	0,31	0,41
	1	252184_at	AT3G50660	steroid 22-alpha-hydroxylase (CYP90B1) (DWF4)	0,47	0,47	0,02	0,02
45	1	263002_at	AT1G54200	expressed protein	1,26	1,16	2,19	2,06
	1	257642_at	AT3G25710	basic helix-loop-helix (bHLH) family protein	0,48	1,06	0,10	0,53
50	1	247933_at	AT5G56980	expressed protein	0,43	0,75	0,22	0,20
	1	261772_at	AT1G76240	expressed protein,	0,40	0,57	0,07	0,16
	1	260034_at	AT1G68810	basic helix-loop-helix (bHLH) family protein	0,80	1,11	0,41	0,83
	1	258091_at	AT3G14560	expressed protein	0,59	0,81	0,35	0,50
55	1	264091_at	AT1G79110	expressed protein	0,66	1,09	0,45	0,77
	1	254024_at	AT4G25780	pathogenesis-related protein	0,12	0,74	0,18	0,26

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	Cluster	Affymetrix no.	Accession no.	Annotation	BIK	BL	BIK	BL
5	1	266799_at	AT2G22860	phytosulfokines 2 (PSK2)	0,68	0,86	0,19	0,44
	1	252387_at	AT3G47800	aldose 1-epimerase family protein	0,91	1,06	0,57	0,26
	1	258196_at	AT3G13980	expressed protein	0,25	0,71	0,08	0,45
	1	262531_at	AT1G17230	leucine-rich repeat family protein	1,09	1,08	0,22	0,38
10	1	249467_at	AT5G39610	no apical meristem (NAM) family protein	0,72	0,65	0,35	0,22
	1	246495_at	AT5G16200	50S ribosomal protein-related	0,37	0,70	0,10	0,20
15	1	245136_at	AT2G45210	auxin-responsive protein-related	0,34	0,77	0,24	0,21
	1	258021_at	AT3G19380	U-box domain-containing protein	0,67	0,70	0,48	0,27
	1	247754_at	AT5G59080	expressed protein	0,46	0,62	0,40	0,53
	1	245325_at	AT4G14130	xyloglucan:xyloglucosyl transferase	0,37	0,85	0,02	0,08
20	1	261700_at	AT1G32690	expressed protein	0,38	0,92	0,26	0,48
	1	259751_at	AT1G71030	Encodes a putative myb family transcription factor	0,92	0,84	0,46	0,13
25	1	262259_s_at	AT1G53870; AT1G53890	[AT1G53870, expressed protein	0,43	0,89	0,51	0,77
	1	248801_at	AT5G47370	homeobox-leucine zipper protein 2 (HAT2)	0,51	0,94	0,43	0,87
30	1	247074_at	AT5G66590	allergen V5/Tpx-1-related family protein	0,53	1,21	0,31	0,58
	1	267515_at	AT2G45680	TCP family transcription factor	0,50	0,91	0,46	0,82
	1	246063_at	AT5G19340	expressed protein	0,83	0,68	0,23	0,39
	1	266150_s_at	AT2G12290; AT4G19700	[AT2G12290, expressed protein];[AT4G19700, expressed protein]	0,38	0,80	0,53	0,55
35	1	245276_at	AT4G16780	homeobox-leucine zipper protein 4 (HAT4) / HD-ZIP protein	0,54	0,62	0,42	0,51
40	1	261597_at	AT1G49780	U-box domain-containing protein	0,71	0,53	0,56	0,28
	1	255538_at	AT4G01680	myb family transcription factor (MYB55)	0,21	0,51	0,14	0,14
	1	245439_at	AT4G16670	expressed protein	0,79	0,78	0,34	0,35
	1	251839_at	AT3G54950	patatin-related,	0,58	0,86	0,43	0,39
45	1	258432_at	AT3G16570	rapid alkalization factor (RALF) family protein	0,54	0,88	0,36	0,87
	1	251827_at	AT3G55120	chalcone-flavanone isomerase	0,94	0,98	0,39	0,68
	1	259982_at	AT1G76410	zinc finger (C3HC4-type RING finger)	0,54	1,61	0,16	0,43
50	1	245229_at	AT4G25620	hydroxyproline-rich glycoprotein family protein	0,51	0,87	0,49	0,86
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	Cluster	Affymetrix no.	Accession no.	Annotation	BIK	BL	BIK	BL
5	1	255177_at	AT4G08040	1-aminocyclopropane-1-carboxylate synthase, putative	0,08	0,13	0,02	0,02
	2	258075_at	AT3G25900	homocysteine S-methyltransferase 1 (HMT-1)	1,32	1,17	2,67	2,22
10	2	246584_at	AT5G14730	expressed protein [AT2G40340, encodes a member of the DREB subfamily A-2 of ERF/AP2	0,38	0,80	0,53	0,55
15	2	263823_s_at	AT2G40340; AT2G40350	ERF/AP2	1,40	1,16	3,20	1,68
	2	245272_at	AT4G17250	expressed protein	1,20	0,86	3,32	1,82
	2	245119_at	AT2G41640	expressed protein	1,96	0,84	2,53	1,43
20	2	266693_at	AT2G19800	expressed protein	2,29	1,20	2,22	1,78
	2	254204_at	AT4G24160	hydrolase, alpha/beta fold family protein	1,42	1,13	2,29	1,14
	2	248732_at	AT5G48070	putative xyloglucan endotransglycosylase/hydrolase	1,48	1,12	3,63	1,24
25	2	262072_at	AT1G59590	expressed protein	1,99	0,95	2,08	1,03
	2	267592_at	AT2G39710	aspartyl protease family protein	2,42	1,25	2,52	1,00
	2	248164_at	AT5G54490	calcium-binding EF-hand protein, putative	0,73	0,37	3,06	1,75
30	2	251910_at	AT3G53810	lectin protein kinase	2,28	0,80	2,23	1,19
	2	249188_at	AT5G42830	transferase family protein	1,03	0,87	2,74	0,62
	2	248134_at	AT5G54860	integral membrane transporter family protein	1,69	0,92	2,01	0,82
35	2	245041_at	AT2G26530	expressed protein	1,24	0,41	1,99	0,86
	2	261648_at	AT1G27730	zinc finger (C2H2 type) family protein	0,56	0,18	1,06	0,71
	2	267028_at	AT2G38470	WRKY family transcription factor	2,02	0,38	1,34	0,57
40	2	253044_at	AT4G37290	expressed protein	0,95	0,90	2,45	0,40
	2	255502_at	AT4G02410	lectin protein kinase family protein	2,60	0,66	1,55	0,59
	2	249494_at	AT5G39050	transferase family protein	1,49	1,35	1,83	0,62
45	2	245369_at	AT4G15975	zinc finger (C3HC4-type RING finger) family protein	2,33	1,10	3,28	1,05
	2	249558_at	AT5G38310	expressed protein	1,00	0,98	1,88	0,93
	2	258282_at	AT3G26910	hydroxyproline-rich glycoprotein family protein	2,49	1,08	1,85	0,60
50	2	252501_at	AT3G46880	expressed protein, short-chain	1,50	0,84	1,51	0,60
	2	266265_at	AT2G29340	dehydrogenase/reductase (SDR) family protein	1,55	0,85	3,73	1,29
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	Cluster	Affymetrix no.	Accession no.	Annotation	BIK	BL	BIK	BL
5	2	253737_at	AT4G28703	expressed protein	1,95	0,85	6,14	0,89
	2	255941_at	AT1G20350	mitochondrial import inner membrane translocase subunit	1,00	0,99	2,67	1,08
10	2	256522_at	AT1G66160	U-box domain-containing protein,	2,03	1,10	1,91	1,22
	2	264746_at	AT1G62300	WRKY family transcription factor	1,58	0,63	0,71	0,21
	2	260602_at	AT1G55920	serine O- acetyltransferase	2,19	1,04	2,16	1,01
15	2	245662_at	AT1G28190	expressed protein	2,79	0,66	1,65	0,58
	2	251039_at	AT5G02020	expressed protein	1,01	0,99	2,29	0,96
	2	256337_at	AT1G72060	expressed protein	2,19	0,96	5,97	0,92
	2	246858_at	AT5G25930	leucine-rich repeat family protein	2,65	0,98	1,38	0,63
20	2	253414_at	AT4G33050	calmodulin-binding family protein	2,38	0,99	1,58	0,57
	2	249765_at	AT5G24030	C4-dicarboxylate transporter/malic acid transport family protein	2,35	1,43	1,66	1,90
25	2	247617_at	AT5G60270	lectin protein kinase family protein	0,96	1,03	2,56	1,82
	2	254294_at	AT4G23070	rhomboid family protein	2,00	1,02	3,80	2,03
	2	245262_at	AT4G16563	aspartyl protease family protein	1,16	1,44	1,65	2,89
30	2	263652_at	AT1G04330	expressed protein	1,17	1,04	4,41	1,30
	2	264083_at	AT2G31230	encodes a member of the ERF (ethylene response factor)	2,13	1,66	3,17	2,74
35	2	246401_at	AT1G57560	myb family transcription factor (MYB50)	1,19	1,04	2,65	2,45
	2	253828_at	AT4G27970	C4-dicarboxylate transporter/malic acid transport family protein	2,43	1,20	3,04	1,28
40	2	262315_at	AT1G70990	proline-rich family protein	1,70	1,59	3,50	2,22
	2	262124_at	AT1G59660	nucleoporin family proteinmily	1,45	1,67	2,94	3,15
	2	267393_at	AT2G44500	expressed protein	1,26	1,16	2,19	2,06
	2	247543_at	AT5G61600	encodes a member of the ERF (ethylene response factor)	0,53	0,46	2,04	2,06
45	2	246200_at	AT4G37240	expressed protein	0,79	1,23	1,45	2,33
	2	260434_at	AT1G68330	expressed protein	1,76	0,90	2,78	1,74
	2	247610_at	AT5G60630	expressed protein	0,99	0,94	2,20	1,15
	2	249752_at	AT5G24660	expressed protein	2,43	1,33	3,61	2,26
50	2	247625_at	AT5G60200	Dof-type zinc finger domain-containing protein	1,36	0,82	3,85	1,67
	2	254292_at	AT4G23030	MATE efflux protein- related	1,60	1,37	5,25	2,20

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	Cluster	Affymetrix no.	Accession no.	Annotation	BIK	BL	BIK	BL
5	2	263207_at	AT1G10550	xyloglucan:xyloglucosyl transferase	1,26	1,34	1,66	2,19
	2	258367_at	AT3G14370	protein kinase family protein	1,10	2,13	2,16	3,50
	2	263931_at	AT2G36220	expressed protein	1,10	1,18	2,76	2,62
10	2	263150_at	AT1G54050	17.4 kDa class III heat shock protein (HSP17.4-CIII)	1,16	1,09	2,86	3,58
	2	256453_at	AT1G75270	dehydroascorbate reductase	1,16	1,27	2,62	1,66
15	2	251162_at	AT3G63300	expressed protein	1,26	1,02	2,07	1,13
	2	267238_at	AT2G44130	kelch repeat-containing F-box family protein	1,13	1,63	7,72	6,30
	2	251774_at	AT3G55840	expressed protein	1,05	1,06	6,96	7,41
20	3	246781_at	AT5G27350	sugar-porter family protein 1 (SFP1)	1,07	1,11	1,56	2,04
	3	248419_at	AT5G51550	phosphate-responsive 1 family protein	1,02	0,86	0,41	0,37
	3	252970_at	AT4G38850	auxin-responsive protein (SAUR-AC1)	1,00	1,43	2,22	3,03
25	3	252972_at	AT4G38840	auxin-responsive protein (SAUR14)	1,01	1,26	1,95	2,53
	3	265806_at	AT2G18010	auxin-responsive family protein (SAUR10)	1,35	1,60	3,94	9,13
30	3	246926_at	AT5G25240	expressed protein	0,74	1,21	2,16	3,69
	3	245176_at	AT2G47440	DNAJ heat shock N-terminal domain-containing protein	1,07	1,31	1,64	2,23
	3	253736_at	AT4G28780	GDSL-motif lipase/hydrolase family protein	1,00	1,25	1,26	2,29
35	3	263325_at	AT2G04240	zinc finger (C3HC4-type RING finger) family protein	0,77	1,46	1,72	2,81
	3	253103_at	AT4G36110	auxin-responsive protein	1,16	2,35	4,62	7,86
40	3	260287_at	AT1G80440	kelch repeat-containing F-box family protein	1,36	1,71	2,89	2,93
	3	255931_at	AT1G12710	F-box family protein	0,88	1,26	1,41	2,11
	3	254424_at	AT4G21510	F-box family protein	1,03	1,47	2,54	2,09
45	3	261265_at	AT1G26800	zinc finger (C3HC4-type RING finger) family protein,	1,07	1,51	4,39	6,09
	3	267614_at	AT2G26710	cytochrome p450 family (BAS1)	0,72	1,56	2,26	5,42
	3	252965_at	AT4G38860	auxin-responsive protein (SAUR16)	0,79	1,38	2,27	3,40
50	3	262630_at	AT1G06520	Encodes a membrane associated mitochondrial localized protein	1,26	1,57	2,28	3,11
55	3	265732_at	AT2G01300	expressed protein	0,73	1,16	3,12	4,83

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	Cluster	Affymetrix no.	Accession no.	Annotation	BIK	BL	BIK	BL
5	3	255037_at	AT4G09460	myb family transcription factor	1,50	1,30	3,27	2,76
	3	248040_at	AT5G55970	zinc finger (C3HC4-type RING finger) family protein	0,96	1,03	2,56	1,82
10	3	259864_at	AT1G72800	nuM1-related, contains short-chain	1,15	1,08	2,15	2,85
	3	249947_at	AT5G19200	dehydrogenase/reductase (SDR) family protein	1,00	1,22	1,11	2,47
15	3	252173_at	AT3G50650	scarecrow-like transcription factor 7 (SCL7)	1,51	1,17	2,48	1,82
	3	266908_at	AT2G34650	protein kinase PINOID (PID)	1,04	1,54	2,52	3,26
20	3	266447_at	AT2G43290	calmodulin-like protein (MSS3)	0,81	1,24	1,45	2,99
	3	262653_at	AT1G14130	2-oxoglutarate-dependent dioxygenase	1,10	1,26	2,47	1,31
	3	261443_at	AT1G28480	glutaredoxin family protein	1,02	0,99	3,97	1,38
25	3	246464_at	AT5G16980	NADP-dependent oxidoreductase	1,11	1,15	3,07	1,26
	4	247940_at	AT5G57190	phosphatidylserine decarboxylase	1,32	1,04	1,58	0,60
30	4	266835_at	AT2G29990	pyridine nucleotide-disulphide oxidoreductase family protein	1,43	0,94	1,38	0,48
35	4	245051_at	AT2G23320	WRKY family transcription factor	1,41	0,86	1,19	0,50
	4	248686_at	AT5G48540	33 kDa secretory protein-related	2,45	1,01	2,03	0,55
	4	262550_at	AT1G31310	hydroxyproline-rich glycoprotein family protein	1,17	0,85	2,45	0,74
40	4	249480_s_at	AT5G38990; AT5G39000	[AT5G38990, protein kinase family protein	1,40	0,94	2,08	0,74
	4	252230_at	AT3G49810	U-box domain-containing protein	1,29	0,89	1,12	0,56
45	4	250004_at	AT5G18750	DNAJ heat shock N-terminal domain-containing protein	1,91	1,03	1,26	0,56
	4	253573_at	AT4G31020	expressed protein	2,67	1,53	5,81	0,67
50	4	246321_at	AT1G16640	transcriptional factor B3 family protein	1,14	1,02	2,20	0,95
	4	258537_at	AT3G04210	disease resistance protein (TIR-NBS class), putative,	2,18	0,89	3,17	1,00
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	Cluster	Affymetrix no.	Accession no.	Annotation	BIK	BL	BIK	BL
5	4	258651_at	AT3G09920	phosphatidylinositol-4-phosphate 5-kinase family protein	1,56	0,94	1,07	0,42
	4	255933_at	AT1G12750	rhomboid family protein	1,46	1,15	1,60	0,78
	4	257398_at	AT2G01990	expressed protein,	1,89	0,91	2,56	1,06
10	4	249435_at	AT5G39970	expressed protein	1,42	1,08	2,66	0,99
	4	251832_at	AT3G55150	exocyst subunit EXO70 family protein	3,44	0,75	0,48	0,13
	4	261382_at	AT1G05470	endonuclease/exonuclease/phosphatase family protein	1,42	1,04	2,59	1,05
15								
	4	254231_at	AT4G23810	WRKY family transcription factor	5,76	0,71	1,00	0,55
	4	246993_at	AT5G67450	zinc finger (C2H2 type) protein 1 (AZF1)	6,17	1,79	1,82	0,89
20	4	252047_at	AT3G52490	heat shock protein-related	1,17	0,93	2,41	1,08
	4	265158_at	AT1G31040	zinc-binding protein-related	1,35	0,88	4,37	1,12
25	4	267171_at	AT2G37590	Dof-type zinc finger domain-containing protein	1,52	0,90	5,81	1,19
	4	255895_at	AT1G18020; AT1G17990	[AT1G18020, 12-oxophytodienoate reductase,	1,31	0,80	1,78	0,50
30	4	257805_at	AT3G18830	polyol/cyclitol/monosaccharide-H+-symporte	1,64	1,11	2,60	1,14
	4	253779_at	AT4G28490	leucine-rich repeat transmembrane protein kinase, putative	1,20	1,00	2,30	0,78
35								
	4	264056_at	AT2G28510	Dof-type zinc finger domain-containing protein	1,28	0,94	1,19	0,51
40								
	5	252296_at	AT3G48970	copper-binding family protein	0,40	0,97	0,38	0,59
	5	254809_at	AT4G12410	auxin-responsive family protein	0,66	1,02	0,31	0,44
45	5	258189_at	AT3G17860	expressed protein	0,78	1,08	0,45	0,74
	5	250598_at	AT5G07690	myb family transcription factor (MYB29)	0,58	0,82	0,27	0,40
	5	246516_at	AT5G15740	expressed protein	0,78	0,95	0,49	0,69
	5	248190_at	AT5G54120	expressed protein	0,82	1,04	0,38	0,94
50	5	253629_at	AT4G30450	glycine-rich protein	0,84	0,88	0,39	0,59
	5	248191_at	AT5G54130	calcium-binding EF hand family protein	0,84	1,03	0,31	0,88
	5	257746_at	AT3G29200	chorismate mutase, chloroplast (CM1)	0,83	0,89	0,49	0,66
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	Cluster	Affymetrix no.	Accession no.	Annotation	BIK	BL	BIK	BL
5	5	247774_at	AT5G58660	oxidoreductase, 20G-Fe (II) oxygenase family protein	1,15	1,08	2,15	2,85
	5	253483_at	AT4G31910	transferase family protein	1,46	1,15	1,60	0,78
	5	252646_at	AT3G44610	protein kinase family protein	0,83	1,05	0,38	1,03
10	5	257066_at	AT3G18280	protease inhibitor/seed storage/lipid transfer protein	1,03	1,06	0,39	0,92
	5	250907_at	AT5G03670	expressed protein	0,61	0,60	0,32	0,37
15	5	253617_at	AT4G30410	expressed protein	0,94	0,97	0,45	0,45
	5	247177_at	AT5G65300	expressed protein	0,31	0,25	0,25	0,36
	5	245885_at	AT5G09440	phosphate-responsive protein, putative	0,38	0,58	0,13	0,13
	5	251072_at	AT5G01740	expressed protein,	0,33	0,53	0,34	0,47
20	6	250012_x_at	AT5G18060	auxin-responsive protein	1,00	0,76	0,46	0,28
	6	259784_at	AT1G29450	auxin-responsive protein	0,99	0,75	0,61	0,37
	6	259783_at	AT1G29510	auxin-responsive protein	1,02	0,77	0,50	0,36
	6	259773_at	AT1G29500	auxin-responsive protein	1,01	0,79	0,61	0,39
25	6	257506_at	AT1G29440	auxin-responsive family protein	0,96	0,69	0,47	0,34
	6	255403_at	AT4G03400	auxin-responsive GH3 family protein	0,81	0,59	0,77	0,48
	6	256528_at	AT1G66140	zinc finger (C2H2 type) family protein	0,87	0,72	0,61	0,46
30	6	248282_at	AT5G52900	expressed protein	1,70	0,74	0,96	0,50
	6	261768_at	AT1G15550	gibberellin 3-beta-dioxygenase	1,12	0,77	1,17	0,52
	6	259787_at	AT1G29460	auxin-responsive protein	1,23	0,75	0,44	0,28
35	6	265877_at	AT2G42380	bZIP transcription factor family protein	1,65	0,81	0,80	0,39
	6	245479_at	AT4G16140	proline-rich family protein	1,16	0,83	1,51	0,75
	6	262040_at	AT1G80080	leucine-rich repeat family protein	1,56	0,77	2,07	0,39
40	6	261203_at	AT1G12845	expressed protein	1,66	0,98	2,71	0,47
	6	262543_at	AT1G34245	expressed protein	1,69	1,14	5,18	0,67
	6	253255_at	AT4G34760	auxin-responsive family protein	1,80	0,81	1,61	0,62
45	7	263126_at	AT1G78460	SOUL heme-binding family protein	1,01	0,88	2,50	1,49
	7	253281_at	AT4G34138	UDP-glucuronosyl/UDP-glucosyl transferase family protein	1,52	1,17	3,16	1,74
50	7	256818_at	AT3G21420	oxidoreductase, 20G-Fe (II) oxygenase family protein	1,13	1,11	2,39	1,29
	7	261023_at	AT1G12200	flavin-containing monooxygenase family protein	1,44	1,11	2,20	1,40
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	Cluster	Affymetrix no.	Accession no.	Annotation	BIK	BL	BIK	BL
5	7	259875_s_at	AT1G76690; AT1G76680	[AT1G76690, 12-oxophytodienoate reductase (OPR2)	1,95	0,85	6,14	0,89
	7	247444_at	AT5G62630	expressed protein	1,62	1,26	2,28	1,26
	7	255443_at	AT4G02700	sulfate transporter	1,76	1,69	2,16	1,20
10	7	264279_s_at	AT1G78820; AT1G78630	[AT1G78820, curculin-like (mannose-binding) lectin family protein	1,27	0,88	1,84	0,86
	7	245543_at	AT4G15260	UDP-glucuronosyl/UDP-glucosyl transferase family protein	1,41	1,01	1,52	0,67
15								
	8	255926_at	AT1G22190	AP2 domain-containing transcription factor	0,45	0,83	0,50	0,89
	8	259364_at	AT1G13260	DNA-binding protein RAV1 (RAV1)	0,32	0,61	0,46	0,67
20	8	259985_at	AT1G76620	expressed protein	0,90	0,94	0,28	0,37
	8	266124_at	AT2G45080	cyclin family protein	0,77	0,84	0,10	0,27
	8	250537_at	AT5G08565	expressed protein	0,87	1,06	0,41	0,78
	8	262050_at	AT1G80130	expressed protein	0,90	1,04	0,18	0,36
25	8	260727_at	AT1G48100	glycoside hydrolase family 28 protein	0,92	1,06	0,38	1,16
	8	256762_at	AT3G25655	expressed protein	1,00	0,98	0,15	0,12
	9	247704_at	AT5G59510	expressed protein	1,00	0,98	0,60	0,13
30	9	250493_at	AT5G09800	U-box domain-containing protein,	1,04	0,80	0,80	0,30
	9	247351_at	AT5G63790	no apical meristem (NAM) family protein,	0,88	0,49	0,69	0,27
	9	253999_at	AT4G26200	1-aminocyclopropane-1-carboxylate synthase	1,11	0,29	0,24	0,11
35	9	261892_at	AT1G80840	WRKY family transcription factor,	0,43	0,27	0,20	0,05
	9	248646_at	AT5G49100	expressed protein	0,72	0,70	0,62	0,38
40	9	251342_at	AT3G60690	auxin-responsive family protein	0,91	0,87	0,29	0,27
	10	252586_at	AT3G45610	Dof-type zinc finger domain	0,94	0,90	2,56	1,05
	10	262505_at	AT1G21680	expressed protein	0,82	1,12	2,01	1,29
45	10	260243_at	AT1G63720	expressed protein	0,92	0,74	0,16	0,17
	10	247601_at	AT5G60850	Dof-type zinc finger domain-containing protein	0,51	1,09	0,64	1,10
50	10	251176_at	AT3G63380	calcium-transporting ATPase	0,85	0,87	0,11	0,10
	10	246389_at	AT1G77380	amino acid carrier	1,32	0,94	0,99	0,40
	10	247205_at	AT5G64890	expressed protein	1,01	0,97	2,18	0,85

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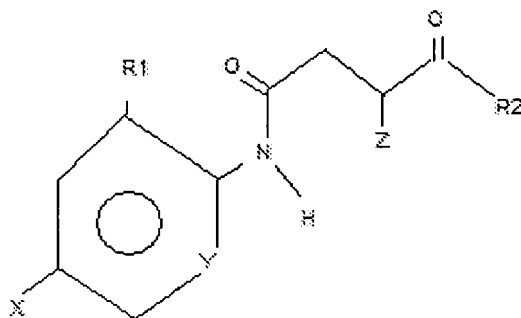
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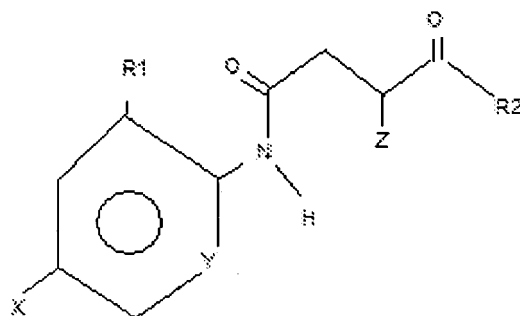
Claims

1. A non-steroidal, monocyclic brassinosteroid mimetic, having the formula



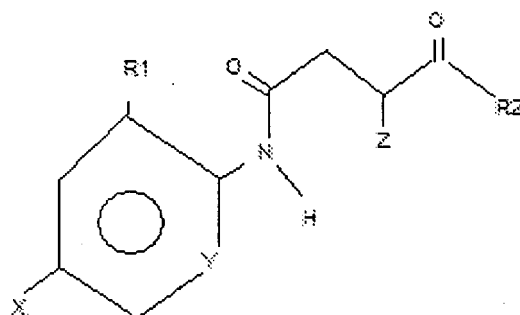
whereby (a) X represents a halogen (b) Y represents nitrogen (c) Z represents hydrogen or a positively charged nitrogen (d) R₁ represents hydrogen or a methyl group and (e) R₂ represents hydrogen, a hydroxyl, methyl or carboxy group, and whereby said non-steroidal monocyclic brassinosteroid mimetic is not 4-[(5-bromo-2-pyridinyl)amino]-4-oxobutanoic acid.

2. A non-steroidal, monocyclic brassinosteroid mimetic according to claim 1 whereby said mimetic is 4-[(5-fluoro-2-pyridinyl)amino]-4-oxobutanoic acid.
3. The use of a non-steroidal, monocyclic brassinosteroid mimetic for promoting plant growth and/or increasing crop yield, said non-steroidal, monocyclic brassinosteroid mimetic having the formula



whereby (a) X represents a halogen (b) Y represents nitrogen (c) Z represents hydrogen or a positively charged nitrogen (d) R₁ represents hydrogen or a methyl group and (e) R₂ represents hydrogen, a hydroxyl, methyl or carboxy group,

4. The use according to claim 3, whereby said non-steroidal, monocyclic compound is selected from a group consisting of 4-[(5-fluoro-2-pyridinyl)amino]-4-oxobutanoic acid, 4-[(5-chloro-2-pyridinyl)amino]-4-oxobutanoic acid, 4-[(5-bromo-2-pyridinyl)amino]-4-oxobutanoic acid and 4-[(5-iodo-2-pyridinyl)amino]-4-oxobutanoic acid.
5. A composition for promoting plant growth, comprising a non-steroidal, monocyclic brassinosteroid mimetic according to claim 1 or 2.
6. A method for promoting plant growth and/or increasing crop yield by applying to the plant an effective amount of a non-steroidal, monocyclic brassinosteroid mimetic having the formula

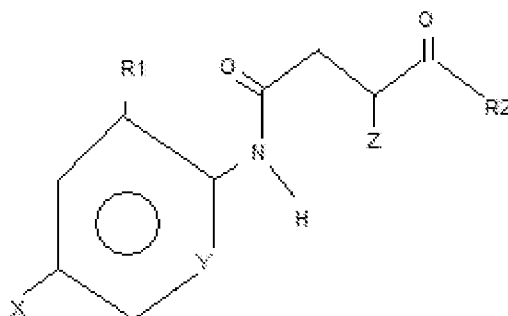


whereby (a) X represents a halogen (b) Y represents nitrogen (c) Z represents hydrogen or a positively charged nitrogen (d) R₁ represents hydrogen or a methyl group and (e) R₂ represents hydrogen, a hydroxyl, methyl or carboxy group.

7. The method of claim 6, whereby said non-steroidal, monocyclic compound is selected from a group consisting of 4-[(5-fluoro-2-pyridinyl)amino]-4-oxobutanoic acid, 4-[(5-chloro-2-pyridinyl)amino]-4-oxobutanoic acid, 4-[(5-bromo-2-pyridinyl)amino]-4-oxobutanoic acid and 4-[(5-iodo-2-pyridinyl)amino]-4-oxobutanoic acid.

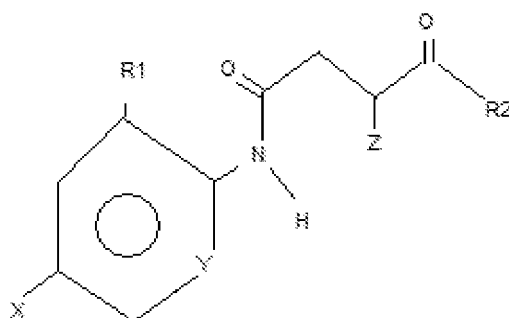
Patentansprüche

1. Nichtsteroides, monocyclisches Brassinosteroid-Mimetikum mit der Formel



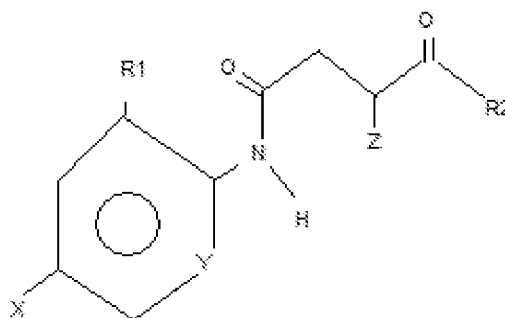
wobei (a) X für ein Halogen steht, (b) Y für Stickstoff steht, (c) Z für Wasserstoff oder einen positiv geladenen Stickstoff steht, (d) R₁ für Wasserstoff oder eine Methylgruppe steht und (e) R₂ für Wasserstoff, eine Hydroxyl-, Methyl- oder Carboxygruppe steht und wobei es sich bei dem nichtsteroiden, monocyclischen Brassinosteroid-Mimetikum nicht um 4-[(5-Brom-2-pyridinyl)amino]-4-oxobutansäure handelt.

2. Nichtsteroides, monocyclisches Brassinosteroid-Mimetikum nach Anspruch 1, wobei es sich bei dem Mimetikum um 4-[(5-Fluor-2-pyridinyl)amino]-4-oxobutansäure handelt.
3. Verwendung eines nichtsteroiden, monocyclischen Brassinosteroid-Mimetikums zur Förderung des Pflanzenwachstums und/oder Erhöhung des Ernteertrags, wobei das nichtsteroides, monocyclische Brassinosteroid-Mimetikum die folgende Formel aufweist:



wobei (a) X für ein Halogen steht, (b) Y für Stickstoff steht, (c) Z für Wasserstoff oder einen positiv geladenen Stickstoff steht, (d) R₁ für Wasserstoff oder eine Methylgruppe steht und (e) R₂ für Wasserstoff, eine Hydroxyl-, Methyl- oder Carboxygruppe steht.

4. Verwendung nach Anspruch 3, wobei die nichtsteroides, monocyclische Verbindung aus einer Gruppe bestehend aus 4-[(5-Fluor-2-pyridinyl)amino]-4-oxobutansäure, 4-[(5-Chlor-2-pyridinyl)amino]-4-oxobutansäure, 4-[(5-Brom-2-pyridinyl)amino]-4-oxobutansäure und 4-[(5-Iod-2-pyridinyl)amino]-4-oxobutansäure ausgewählt ist.
5. Zusammensetzung zur Förderung des Pflanzenwachstums, die ein nichtsteroides, monocyclisches Brassinosteroid-Mimetikum nach Anspruch 1 oder 2 umfasst.
6. Verfahren zur Förderung des Pflanzenwachstums und/oder Erhöhung des Ernteertrags durch Anwenden einer wirksamen Menge eines nichtsteroiden, monocyclischen Brassinosteroid-Mimetikums auf die Pflanze, das die folgende Formel aufweist:

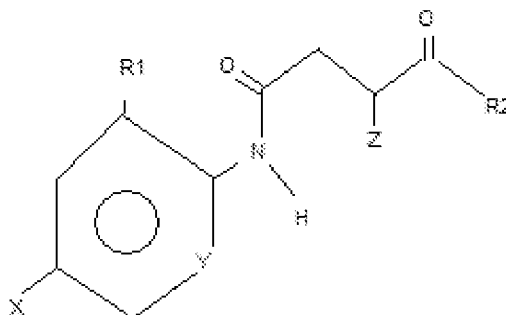


wobei (a) X für ein Halogen steht, (b) Y für Stickstoff steht, (c) Z für Wasserstoff oder einen positiv geladenen Stickstoff steht, (d) R₁ für Wasserstoff oder eine Methylgruppe steht und (e) R₂ für Wasserstoff, eine Hydroxyl-, Methyl- oder Carboxygruppe steht.

7. Verfahren nach Anspruch 6, wobei die nichtsteroiden, monocyclische Verbindung aus einer Gruppe bestehend aus 4-[(5-Fluor-2-pyridinyl)amino]-4-oxobutansäure, 4-[(5-Chlor-2-pyridinyl)amino]-4-oxobutansäure, 4-[(5-Brom-2-pyridinyl)amino]-4-oxobutansäure und 4-[(5-Iod-2-pyridinyl)amino]-4-oxobutansäure ausgewählt ist.

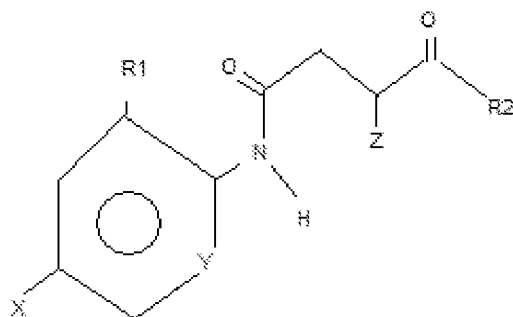
Revendications

1. Mimétique de brassinostéroïde monocyclique non stéroïdien ayant la formule



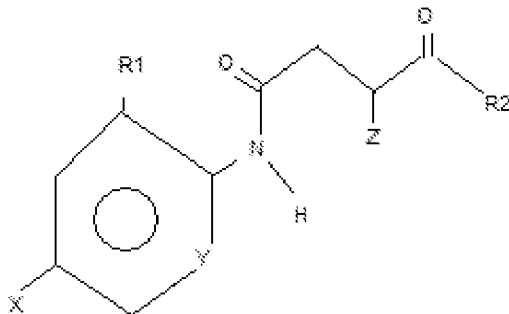
, dans laquelle (a) X représente un halogène, (b) Y représente de l'azote, (c) Z représente de l'hydrogène ou un azote à charge positive, (d) R₁ représente de l'hydrogène ou un groupe méthyle et (e) R₂ représente de l'hydrogène, un groupe hydroxyle, méthyle ou carboxy, et dans lequel ledit mimétique de brassinostéroïde monocyclique non stéroïdien n'est pas l'acide 4-[(5-bromo-2-pyridinyl)amino]-4-oxobutanoïque.

2. Mimétique de brassinostéroïde monocyclique non stéroïdien selon la revendication 1, dans lequel ledit mimétique est l'acide 4-[(5-fluoro-2-pyridinyl)amino]-4-oxobutanoïque.
3. Utilisation d'un mimétique de brassinostéroïde monocyclique non stéroïdien pour promouvoir la croissance végétale et/ou augmenter le rendement des cultures, ledit mimétique de brassinostéroïde monocyclique non stéroïdien ayant la formule



, dans laquelle (a) X représente un halogène, (b) Y représente de l'azote, (c) Z représente de l'hydrogène ou un azote à charge positive, (d) R₁ représente de l'hydrogène ou un groupe méthyle et (e) R₂ représente de l'hydrogène, un groupe hydroxyle, méthyle ou carboxy.

4. Utilisation selon la revendication 3, dans laquelle ledit composé monocyclique non stéroïdien est sélectionné parmi un groupe constitué de l'acide 4-[(5-fluoro-2-pyridinyl)amino]-4-oxobutanoïque, l'acide 4-[(5-chloro-2-pyridinyl)amino]-4-oxobutanoïque, l'acide 4-[(5-bromo-2-pyridinyl)amino]-4-oxobutanoïque et l'acide 4-[(5-iodo-2-pyridinyl)amino]-4-oxobutanoïque.
5. Composition pour promouvoir la croissance végétale, comprenant un mimétique de brassinostéroïde monocyclique non stéroïdien selon la revendication 1 ou 2.
6. Procédé pour promouvoir la croissance végétale et/ou augmenter le rendement des cultures en appliquant à la plante une quantité efficace d'un mimétique de brassinostéroïde monocyclique non stéroïdien ayant la formule



, dans laquelle (a) X représente un halogène, (b) Y représente de l'azote, (c) Z représente de l'hydrogène ou un azote à charge positive, (d) R₁ représente de l'hydrogène ou un groupe méthyle et (e) R₂ représente de l'hydrogène, un groupe hydroxyle, méthyle ou carboxy.

7. Procédé selon la revendication 6, dans lequel ledit composé monocyclique non stéroïdien est sélectionné parmi un groupe constitué de l'acide 4-[(5-fluoro-2-pyridinyl)amino]-4-oxobutanoïque, l'acide 4-[(5-chloro-2-pyridinyl)amino]-4-oxobutanoïque, l'acide 4-[(5-bromo-2-pyridinyl)amino]-4-oxobutanoïque et l'acide 4-[(5-iodo-2-pyridinyl)amino]-4-oxobutanoïque.

Figure 1

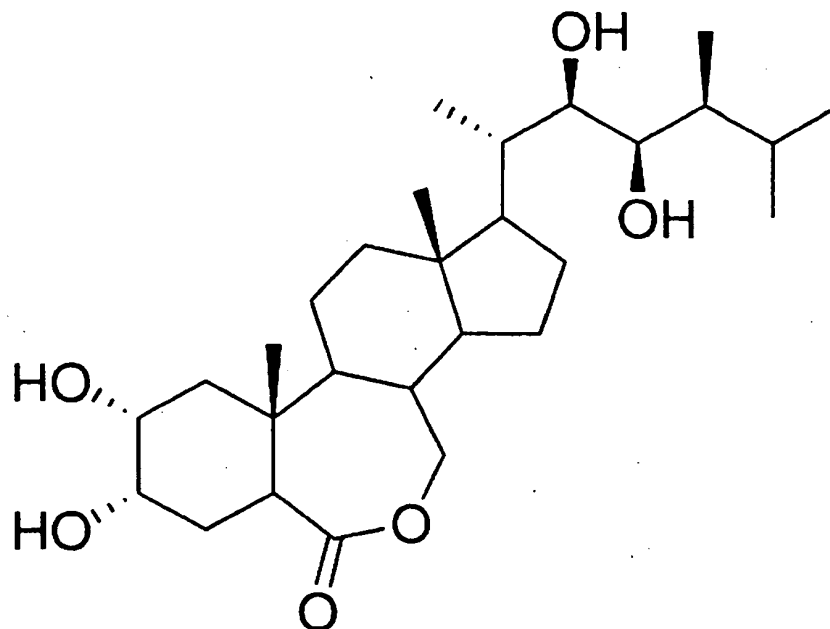


Figure 2 (according to Wang, X. & Chory, J. *Science* 313, 1118-1122 (2006))

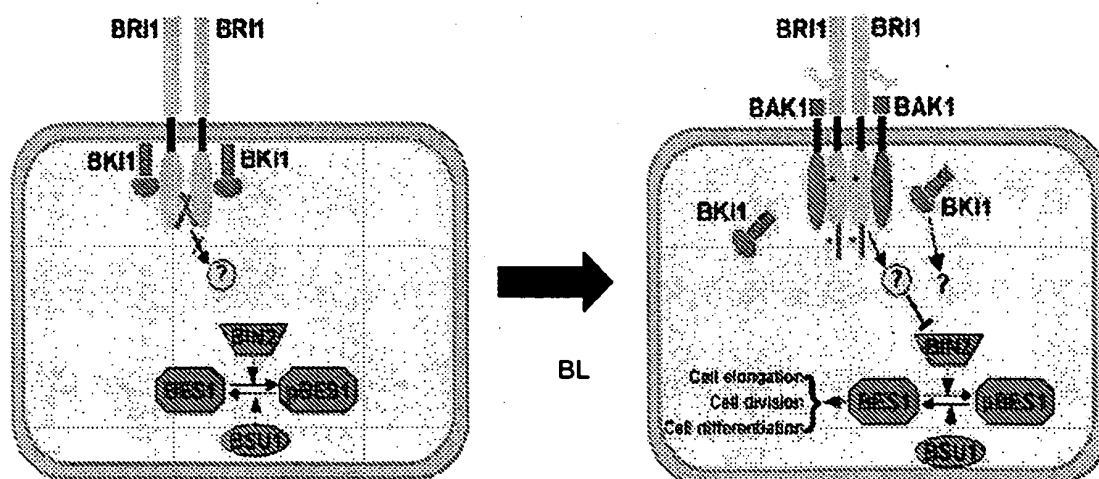
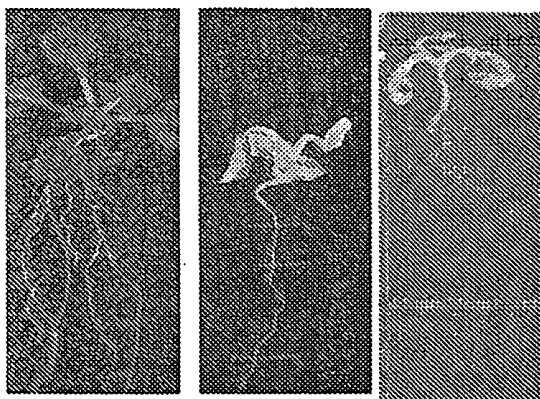


Figure 3

A



B

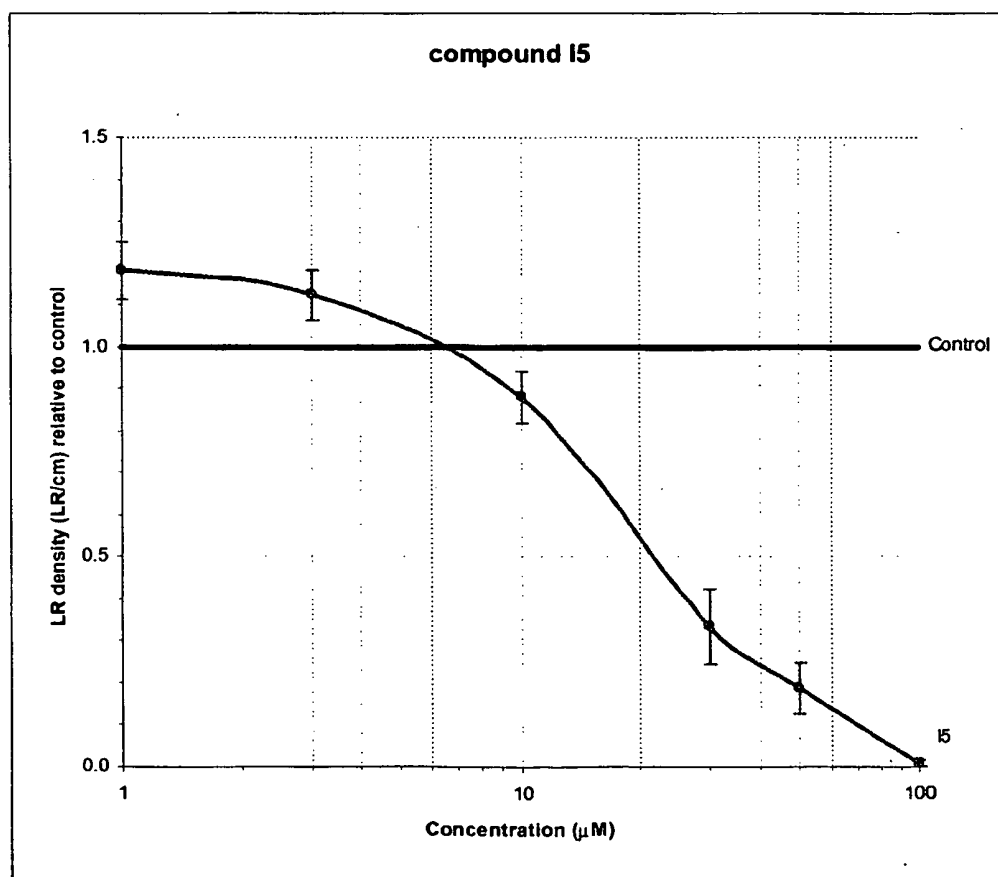


Figure 4

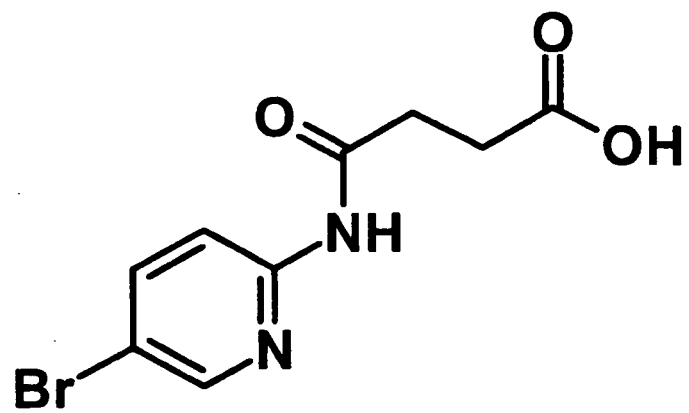


Figure 5

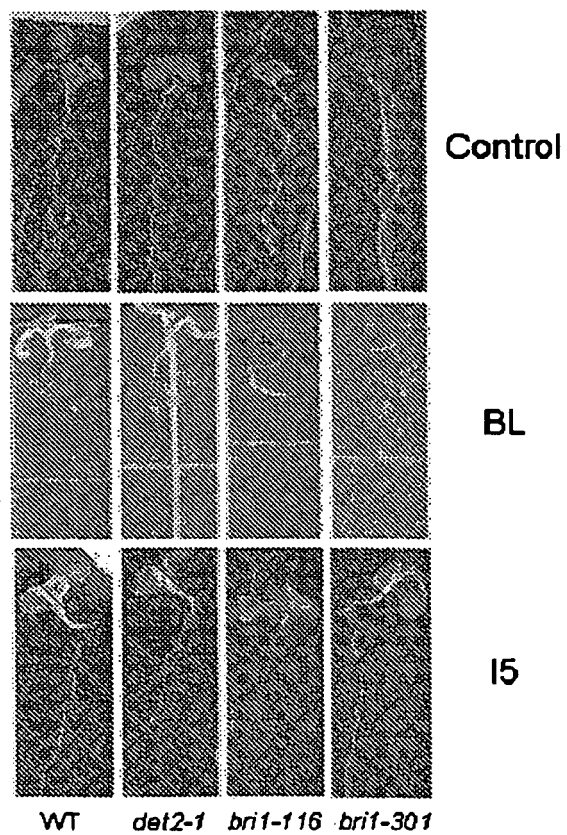


Figure 6

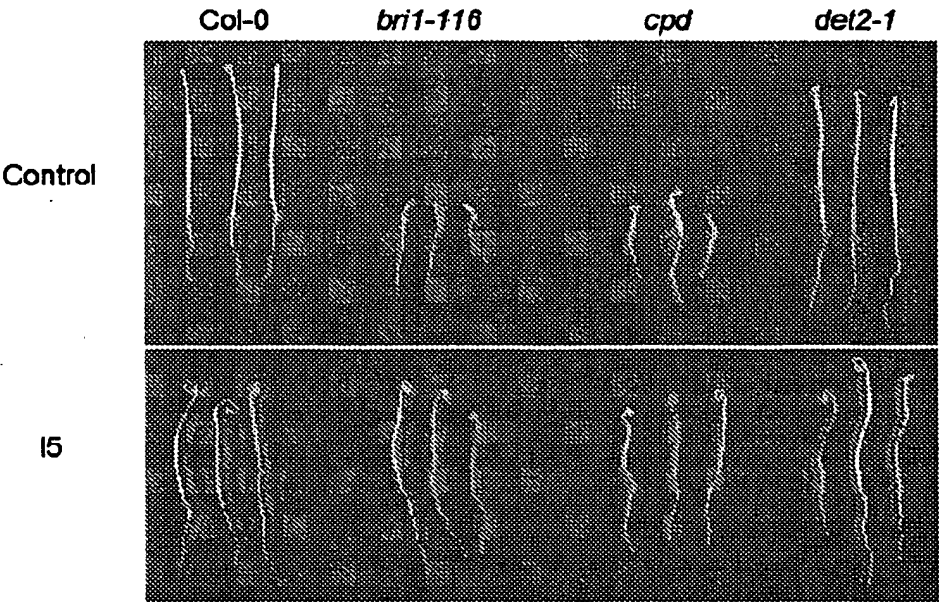


Figure 7

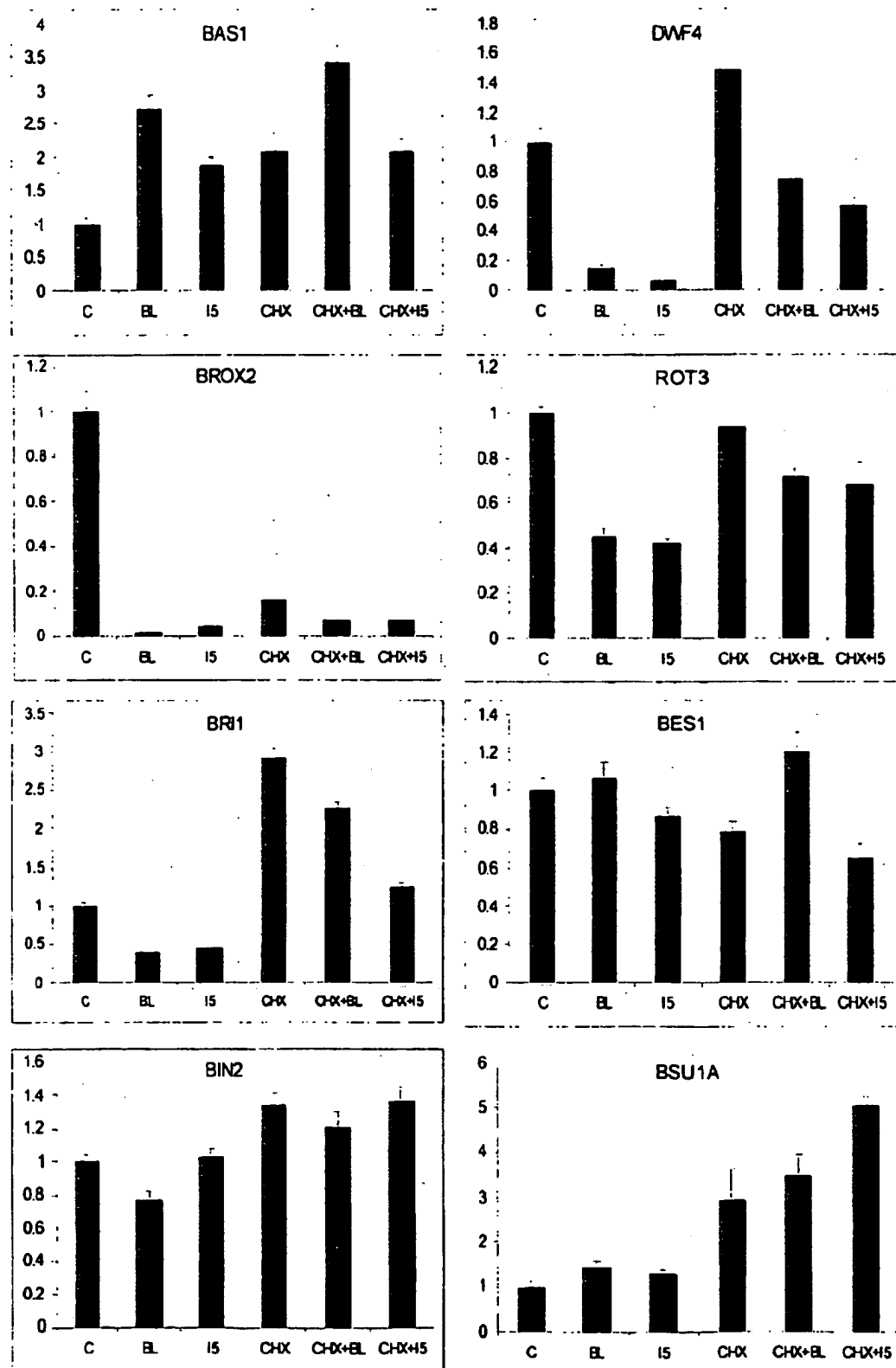


Figure 7 cont.

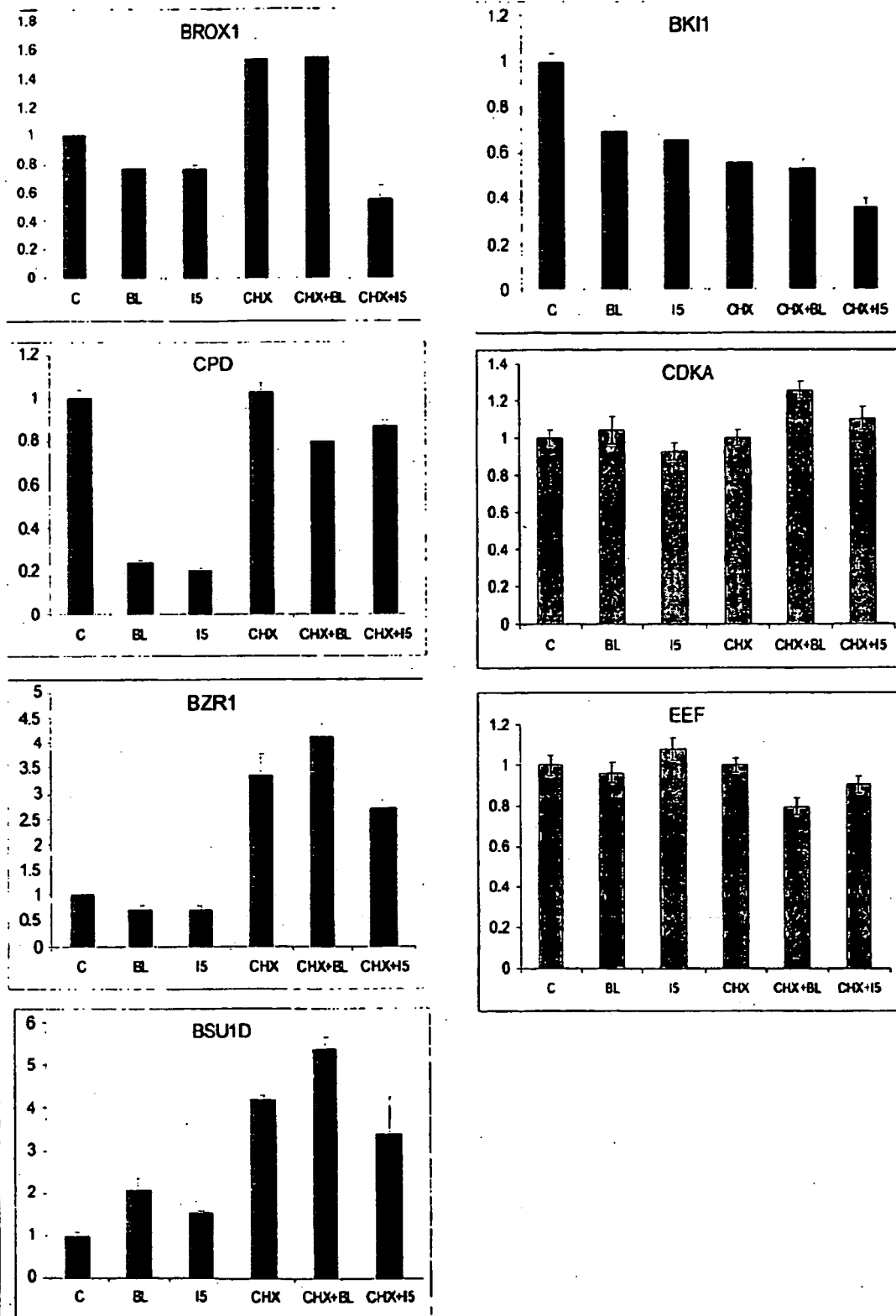


Figure 8 A

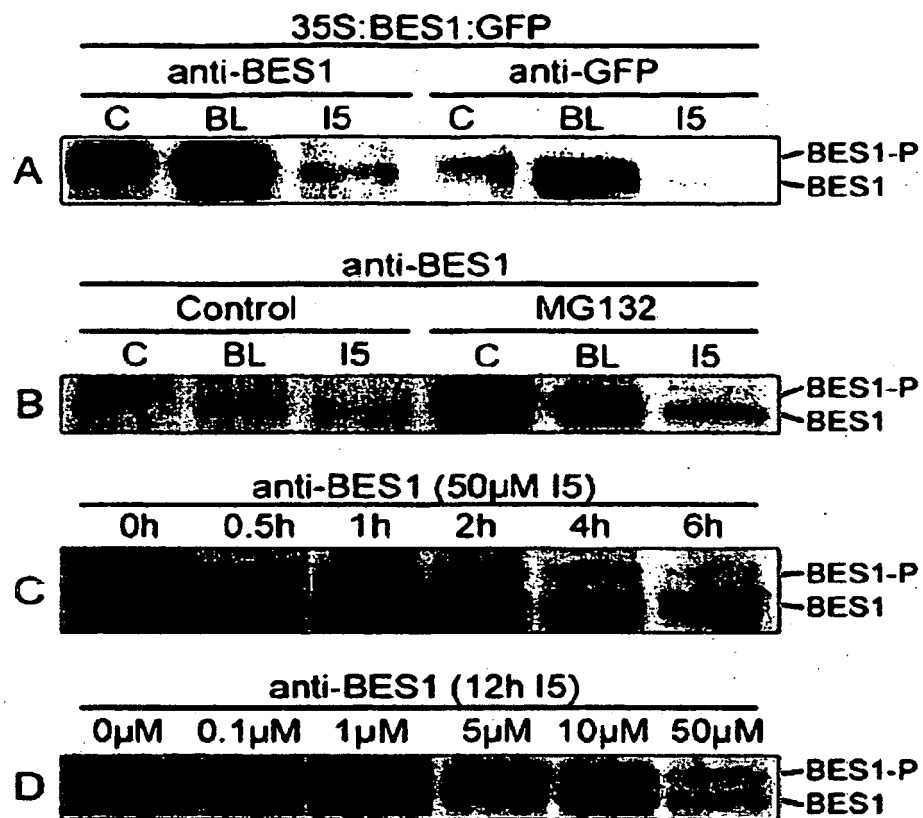


Figure 8B

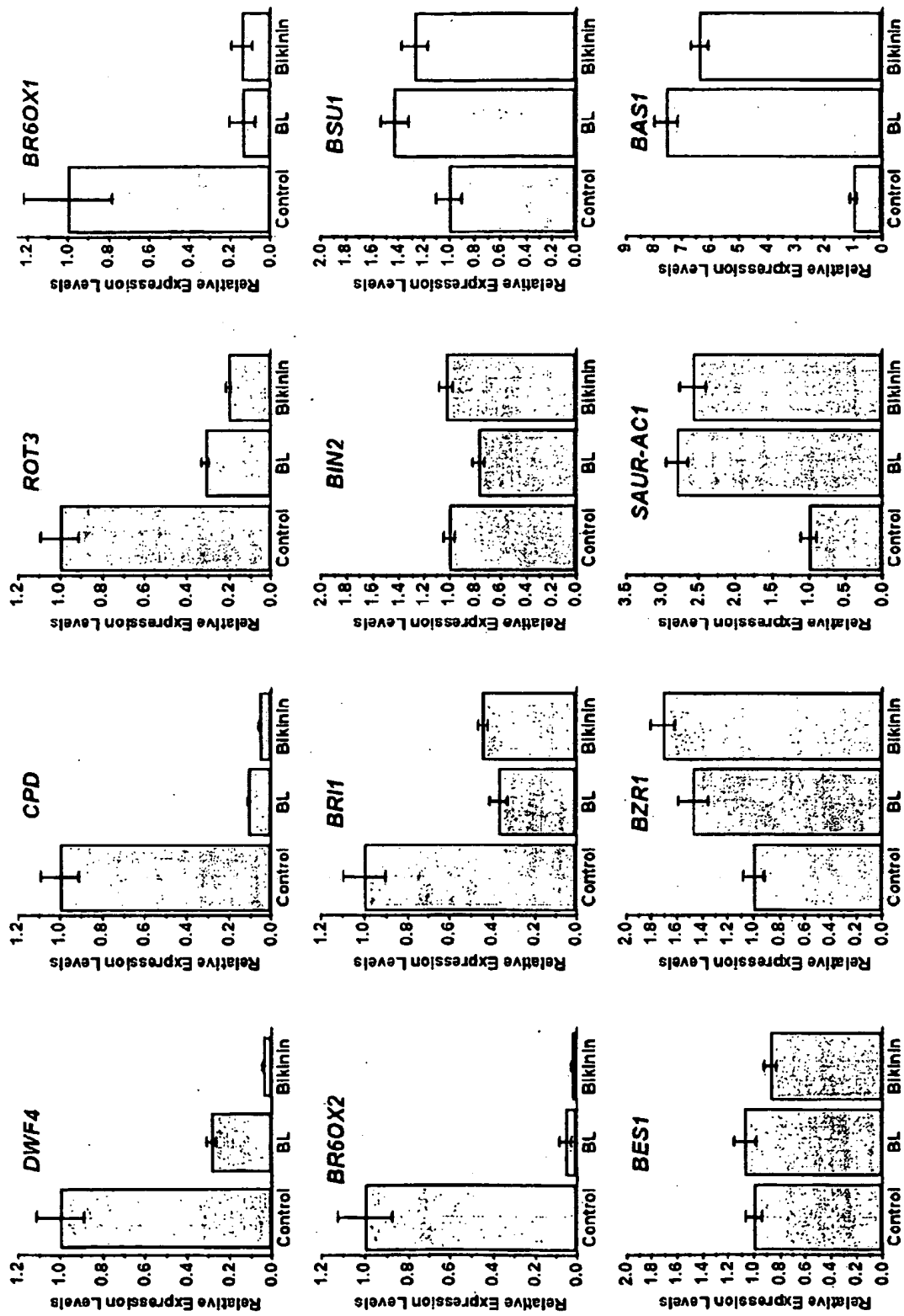
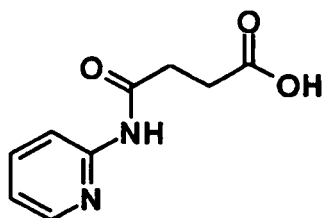
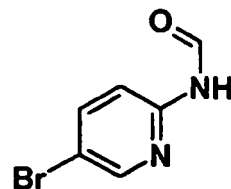


Figure 9

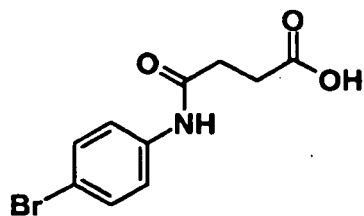
I05 VAR1



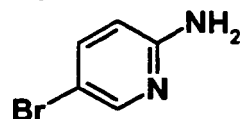
I05 VAR5



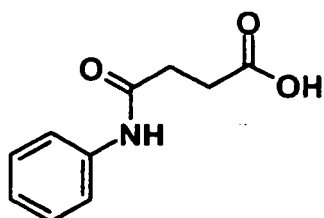
I05 VAR2



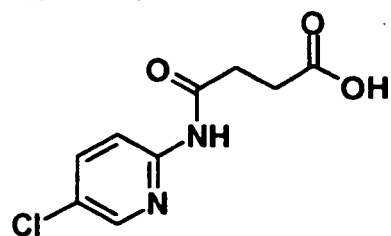
I05 VAR6



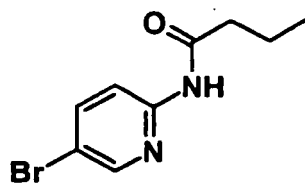
I05 VAR3



I05 VAR7



I05 VAR4



I05 VAR8

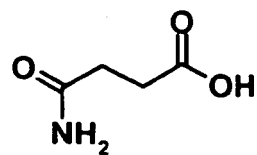


Figure 10

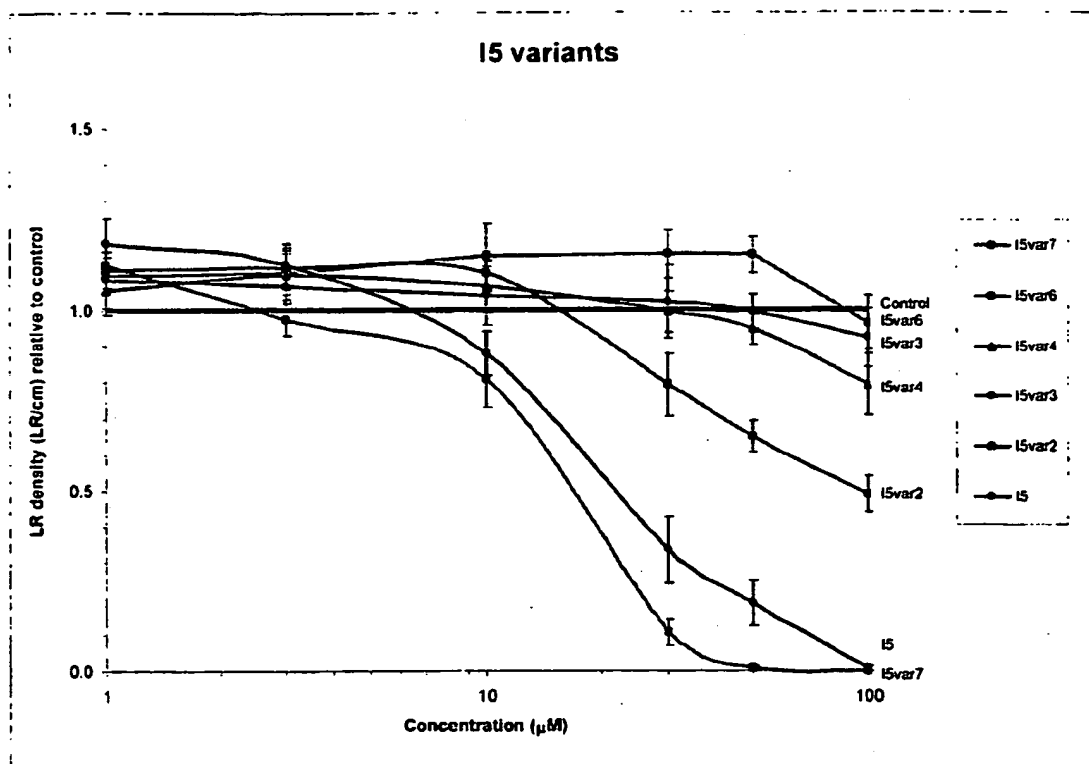
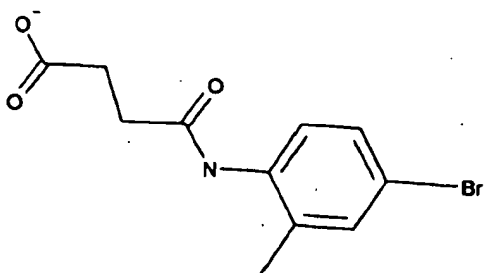
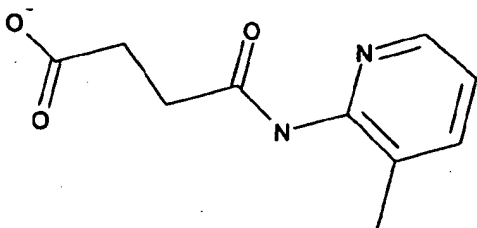


Figure 11



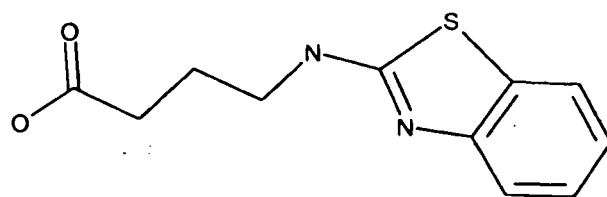
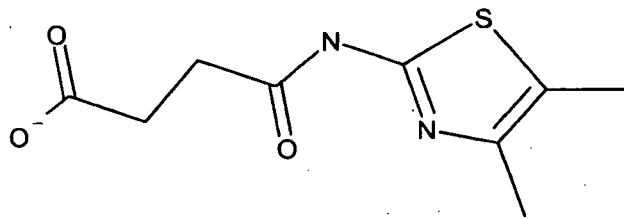
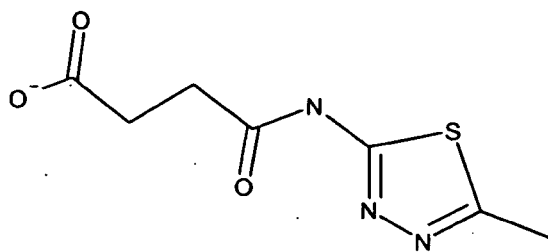
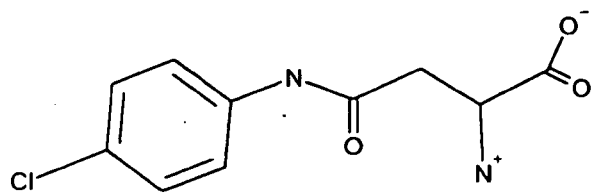


Figure 12

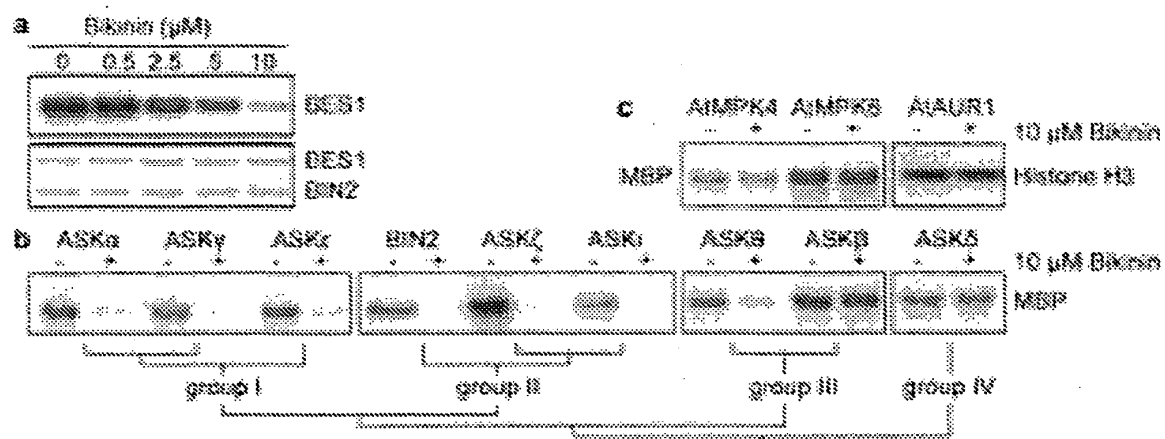


Figure 13

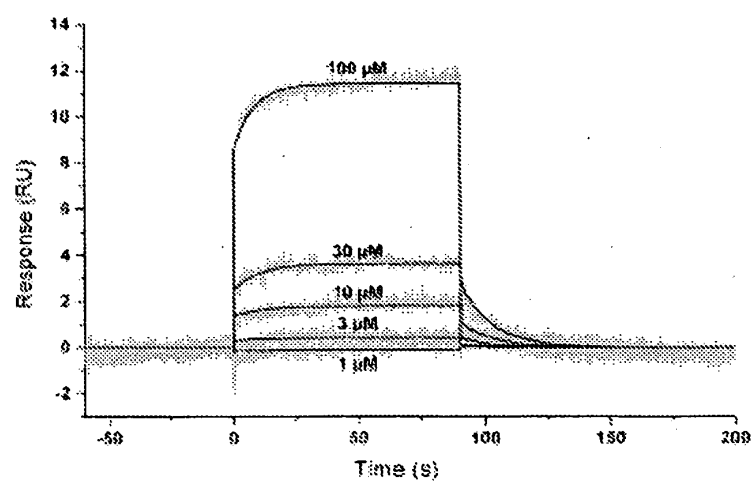


Figure 14

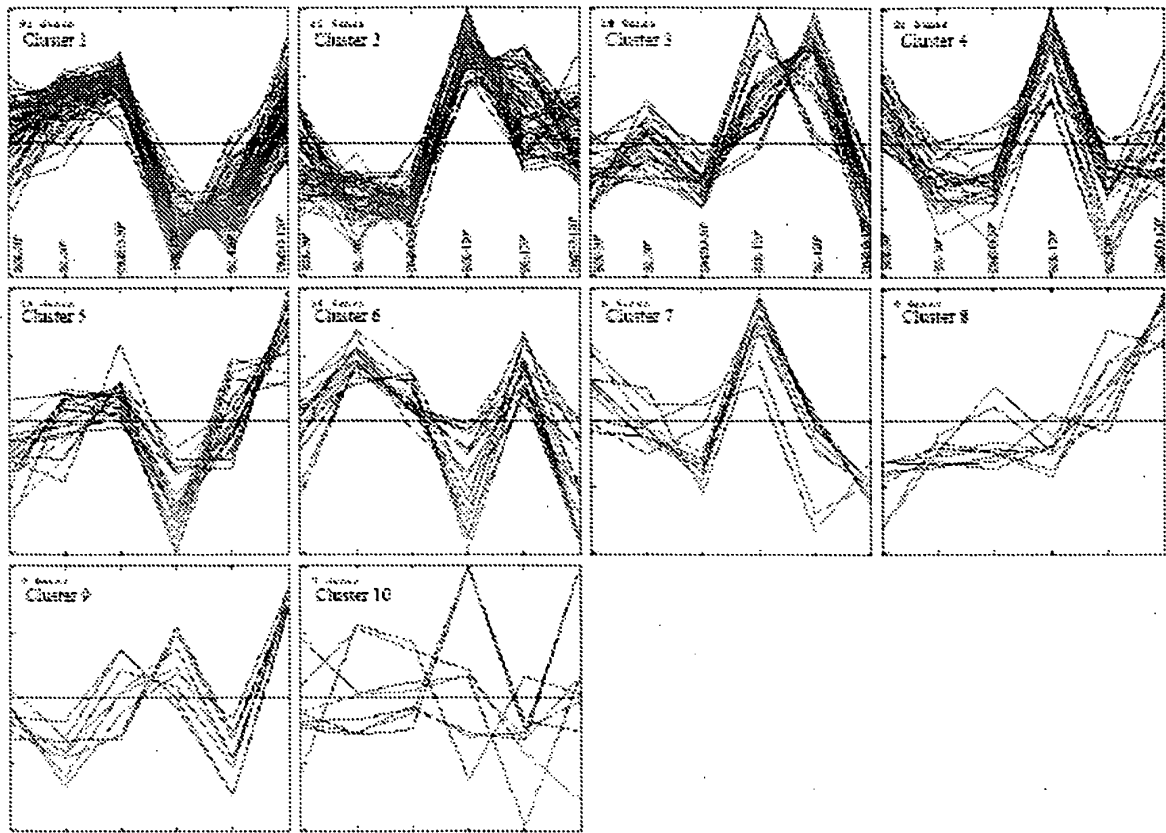


Figure 15

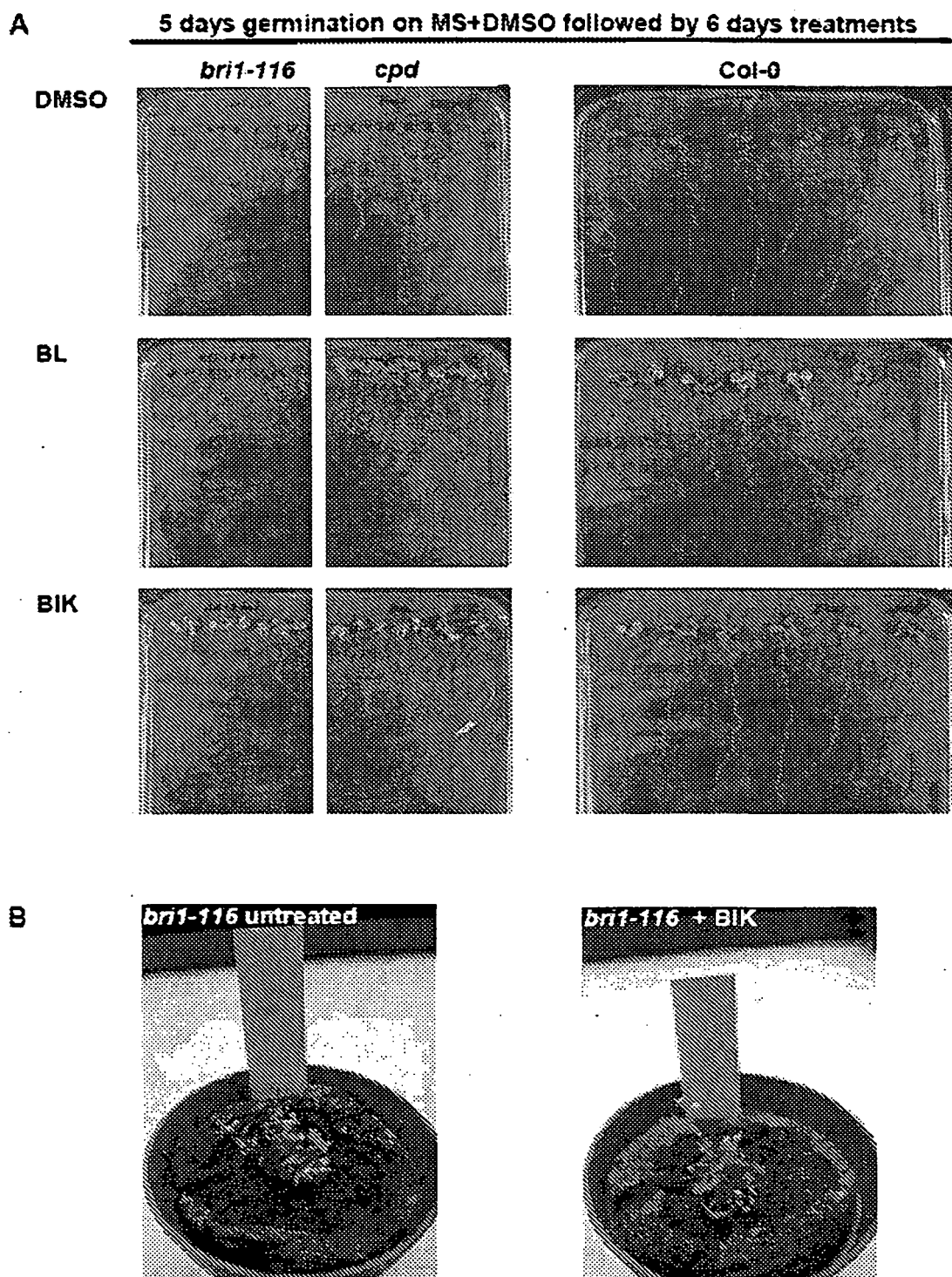
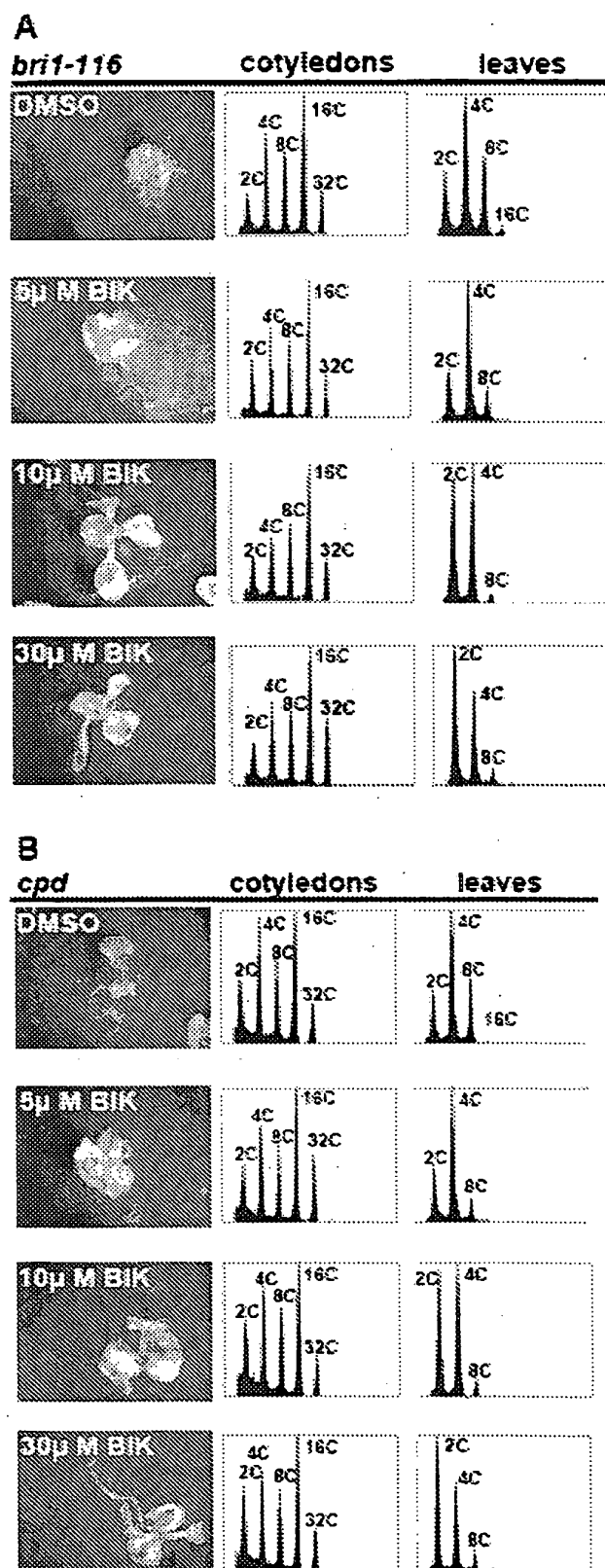


Figure 16



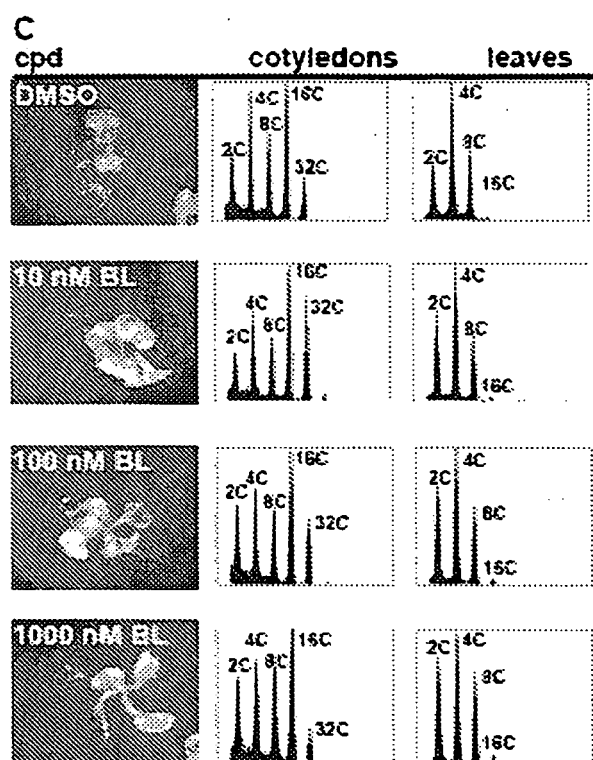
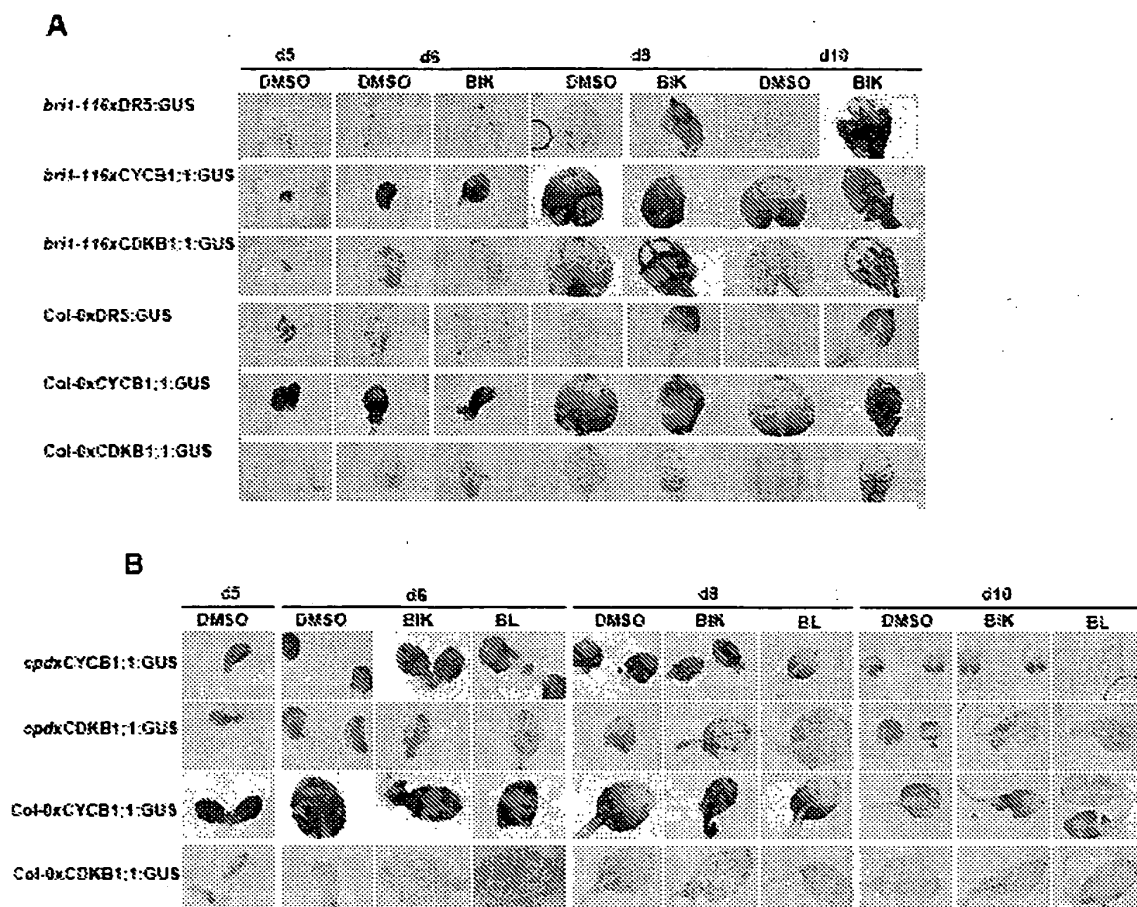


Figure 17



REFERENCES CITED IN THE DESCRIPTION

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